IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Henry Nicolas Jabbour *et al*
Serial No:	10/511,480
Filed:	April 10, 2003
Title:	FP Receptor Antagonists or PGF2 alpha Antagonists for Treating Pathological Conditions of the Uterus
Group Art Unit:	4173
Examiner:	Marcos Sznaidman

DECLARATION OF HENRY NICOLAS JABBOUR UNDER 37 C.F.R. § 1.132

I, Henry Nicolas Jabbour, pursuant to 37 C.F.R. § 1.132, declare:

1. I am one of the inventors of the above-mentioned patent application. I am a Programme Leader and Senior Scientist at the UK Medical Research Council Human Reproductive Science Unit in Edinburgh, Scotland. I have worked in the field of reproductive biology and health for approximately 20 years. My resumé is attached as Annex 1.

2. I have read the Office Action dated January 29, 2008 and I understand that the examiner has rejected Claims 1, 3-5, 9 and 12-13 of the application as failing to comply with the enablement requirement. I am presenting this declaration to demonstrate (1) the availability of FP receptor antagonists, and (2) the credibility of their use in treating pathological conditions of the uterus, and in particular uterine carcinoma, endometriosis and fibroids.

3. FP receptor antagonists are a pharmacologically well-defined class of molecules which were well known by 2002. They typically act by binding to the FP receptor and prevent signalling from that receptor as is described on page 6 of the patent application. Numerous examples of FP receptor antagonists are described in the patent application. For example, on page 2, lines 10-17 reference is made to Griffin *et al* (1999) *J. Pharmacol. Exp. Ther.* **290**, 1278-1284, which describes the selective FP receptor antagonist AL-8810, and to Sharif *et al* (2000) *J. Pharm. Pharmacol.* **52**, 1529-1539, which describes the FP receptor antagonist AL-3138. Dozens of other peptide and non-peptide FP receptor antagonists are described on pages 7 to 11 of the patent application, and many of the references describing these other FP receptor antagonists are incorporated by reference into the specification.

4. Other FP receptors beyond those described in the patent application are known, such as AS604872. AS604872 has been studied by Merck & Co, and in Chollet *et al* (2007) *BMC Pregnancy and Childbirth* 7, S16 it has been proposed to have therapeutic potential for the treatment of preterm labour in which uterine hyperactivity plays a dominant role. Chollet *et al* is enclosed as Annex 2. This study, among others, clearly demonstrates the benefit of targeting the FP receptor – specifically its antagonism – as potential therapy in pathophysiology. While Chollet *et al* does not involve a pathological condition of the uterus as claimed, it certainly confirms the ability to deliver an FP receptor antagonist to achieve therapeutic effect (inhibiting preterm labour). Other molecules that antagonise the FP receptor will be expected to work in the same way ie by blocking the ability of the receptor to be occupied by the natural ligand and to inhibit its ability to signal inside the cell.

5. As is described in the patent application, my fellow inventors and I showed for the first time that there is a higher level of expression of the FP receptor in the uterus during the proliferative phase of the endometrium in the menstrual cycle and that expression in uterine carcinoma tissue is significantly elevated compared with normal uterine tissue. Thus, there is elevated expression of the FP receptor in pathological conditions of the uterus (as is discussed in more detail below). When elevated receptor levels are associated with a pathological condition of the tissue or organ in which the elevated receptor levels are found, a credible pharmacological intervention is to inhibit the function of the overexpressed receptor. For example, in many cancers, cell surface receptors such as EGFR are upregulated as the tissue becomes cancerous/metastatic. It is a conventional treatment to use a molecule that binds to the receptor (such as the monoclonal antibody Cetuximab in the case of EGFR) to prevent stimulation of the receptor by endogenous ligands. Clearly, the overexpression is associated with the pathology, and typically there is additional, undesirable signalling due to the overexpression of the receptor. Antagonism of the receptor is able to reduce the undesirable signalling in the pathological condition, which typically is of therapeutic use.

6. Further work that I have been intimately involved with confirms that FP receptor antagonists are useful in treating pathological conditions of the uterus, as described in the patent application.

7. Attached as Annex 3 is a copy of Sales *et al* (2005) *Cancer Res.* **65**, 7707-7716, which was published after the patent application was filed. I am the corresponding author of this paper.

8. The work in this paper was done under my supervision in my laboratory, and it builds on the work described in the patent application (which shows elevated expression of FP receptor in uterine carcinoma). In particular, the work presented in this paper shows that elevated FP receptor and VEGF expression co-localized in glandular and epithelial cells lining the blood vessels in endometrial (uterine) adenocarcinomas. Furthermore, it shows that PGF2α (the natural ligand of the FP receptor) can cause rapid transphosphorylation and activation of the EGF receptor, and activation of MEK signalling *via* the FP receptor resulting in an increase in VEGF promoter activity, expression of VEGF mRNA and secretion of VEGF protein, all of which are consistent with a role for the FP receptor in stimulating blood vessel formation (angiogenesis) in endometrial (uterine) cancer (see Abstract).

9. These effects of PGF2α on the FP receptor could be abolished by treatment of cells with a specific FP receptor antagonist, AL8810 (see, in particular, page 7711, column 2; and also see Figure 4b, lane 3) and similar effects were found when endometrial adenocarcinoma explants were treated with AL8810 (see, in particular, page 7713, column 2; and also see Figure 6a, lane 3). Consistent with the description in the present application, these results confirm that an FP receptor antagonist can play a direct role in treating a pathological condition of the uterus, such as uterine carcinoma. AL8810 is one of the FP receptor antagonists specifically exemplified in the patent application at page 9, lines 1-7.

10. Attached as Annex 4 are additional data which show that FP receptor expression is consistently higher in the endometrium of women with fibroids, during all phases of the menstrual cycle, than those without fibroids. In this study, which was conducted in my laboratory under my supervision, endometrium was collected from women with fibroids and those without, RNA was extracted from these tissues and then we assessed the level of expression of the FP receptor from the two groups of women by a technique known as reverse transcriptase polymerase chain reaction (this technique allows one to make direct comparisons of the levels of expression of the

receptor in different women). In the endometrium of women with fibroids, the level of expression of the FP receptor was consistently higher. Taking into account our knowledge of the mechanism of action of the FP receptor and its role in exacerbating vascular function, we believe that antagonism of action and signalling of this elevated FP receptor in the endometrium of women with fibroids may be an effective therapeutic intervention strategy that may limit the blood loss that is associated with this pathology. For the reasons discussed above, this gives further credibility that FP receptor antagonists are useful in treating pathological conditions of the uterus, including fibroids.

11. In summary, the data presented in the present application and obtained subsequent to the filing date confirm that several uterine pathological conditions, including endometriosis, uterine carcinoma, and fibroids, all involve enhanced FP receptor expression; and, consistent with the description in the application for treating such uterine pathological conditions, treatment of uterine carcinoma explants with an FP receptor antagonist was shown to be effective in preventing FP receptor mediated expression of pro-angiogenic factors like VEGF. The results therefore confirm the efficacy of treating pathological conditions of the uterus using FP receptor antagonists. Given the demonstrated efficacy of treating uterine carcinoma explants with an FP receptor antagonist, persons of skill in the art would fully appreciate that a female individual having a pathological condition of the uterus, such as uterine carcinoma, endometriosis, uterine fibroids, or any other pathological conditions of the uterus that are associated with abnormal growth of the myometrium or endometrium, can be effectively treated for the condition by administering to the affected individual an FP receptor antagonist.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed at Edinburgh, Scotland

This 24th day of February, 2009

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Henry Nicolas Jabbour

Annex 1: Resume of Henry Nicolas Jabbour

Curriculum Vitae

Name:	Henry Nicolas JABBOUR
Date of Birth:	18 February 1961
Nationality:	Australian and British
Professional address:	MRC Human Reproductive Sciences Unit Centre for Reproductive Biology, The University of Edinburgh Academic Centre, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB United Kingdom Tel: 44 131 242 6220 Fax: 44 131 242 6231 Email: h.jabbour@hrsu.mrc.ac.uk
Current position:	MRC Scientific Staff: non-clinical senior scientist, Band 2. Team leader, MRC Reproductive Biology Unit.
Date of first appointment:	1995
Awarded tenure:	January 2001
Appointment within The University of Edinburgh:	Honorary Fellow, School of Clinical Sciences and Communty Health, College of Medicine and Vetinary Medicine, the University of Edinburgh (2000-present).
University education and degrees awarded:	PhD, The University of Sydney 1989 BSc Agriculture (Distinction), The American University of Beirut 1982 Diploma of Ingenieur Agricole (Distinction), The American University of Beirut 1982

Career since graduation:

1995-present: MRC Scientific Staff: non-clinical senior scientist, Band 2. Team leader, MRC Reproductive Biology Unit.

Honorary Fellow in the School of Clinical Sciences and Community Health in the College of Medicine and Veterinary Medicine of The University of Edinburgh (2000 - present)

Honorary Fellow in the Division of Medical Biochemistry in the Faculty of Medicine of The University of Cape Town (2002-2010)

Affiliated Lecturer, Department of Physiology and Environmental Science, University of Nottingham (October 1995 - September 2001)

Honorary Research Fellow, Institute of Zoology, The Zoological Society of London (January 1996-December 1999)

Visiting Research Fellow, Programme for Population Genetics, Harvard School of Public Health, Harvard University, Boston, MA (nine months period in 1999 supported by a fellowship from Hope International)

1991-1995: Post-doctoral Research Fellow, Institute of Zoology, Regent's Park, London NW1 4RY.

Research Fellow, Molecular Endocrinology Laboratory, INSERM Unite 344, Faculte de Medecine Necker-Enfants Malades, Paris (12 months between 1992-94, partly supported by a postodoctoral fellowship from EMBO, and Travel awards from the BBSRC and The Royal Society)

Honorary Research Fellow, The University of London

1989-1991: Scientist, Ruakura Agricultural Centre, Ministry of Agriculture and Fisheries, New Zealand.

Major research interests:
Many key reproductive events display hallmark signs of inflammation and many reproductive pathologies result from exacerbated inflammation. My research interests are focussed on understanding the role of lipid mediators such as prostaglandins and lipoxins/resolvins in mediating the inflammatory and pro-resolution responses in key reproductive events and how their dysregulated expression/function can lead to benign and neoplastic pathologies of the female reproductive tract. Many of these mediators function by signalling via their receptors, which are members of the 7-transmembrane G-protein coupled receptor family. My research interests also involve unravelling the intracellular signalling of these lipid mediator GPCRs and how they cross talk with each other to influence cell fate and the inflammatory-resolution outcome.
Another programme of research that I have initiated in the last five years has investigated the role of newly identified molecules termed prokineticins as novel regulators of reproductive processes. My research has highlighted that these mediators are important regulators of endometrial receptivity around the time of embryo implantation. Moreover, these factors have been shown to play important roles in the inflammatory events that initiate the onset of labour and we have proposed that premature activation of these mediators and their signalling pathways may be the trigger for the onset of preterm labour. I am currently working with the MRC Drug Discovery Group and MRC

Technology to identify small molecule antagonists. This will prove very useful in elucidating further the role of these novel mediators in key reproductive events such as receptivity, placentation and labour and their pathologies.
These programmes of research involve strong collaborative links with clinician and basic scientists in various departments and centres within The University of Edinburgh and these collaborative links have led to numerous joint publications and grant applications as highlighted in relevant sections within my CV.

Principal research grants:

*University of Edinburgh collaborators are denoted by **

1. Royal Society International Incoming Short Visit grant 2008/R2 awarded to HN Jabbour and A Katz (£4,782). The grant was awarded to conduct collaborative research on role of PGE_2 and seminal plasma PGE_2 in cervical cancer.
2. Project grant (A Horne*, HN Jabbour, G Entrican and HOD Critchley*) from WellBeing of Women. The project is entitled: "Aberrant prokineticin expression as a cause for ectopic pregnancy: the effects of chlamydia trachomatis infection and smoking". (September 2008 – August 2010; £128,445).
3. Project grant (P De Sousa*, S Tomlinson and HN Jabbour) from the European Commission – Seventh framework programme for research and technology development (call identifier: FP7-Health-2007-A). The project is entitled: "Embryonic stem cell-based novel alternative testing strategies". April 2008-March 2011; £683,103).
4. Project grant (HOD Critchley*, HN Jabbour and N Hirani*) from MRC. The project is entitled: "Hypoxia inducible factor (HIF-1α) and endometrial remodelling: relevance to menstrual bleeding". (June 2007- May 2010; £609,033)
5. Small project grant (awarded to F Denison*, G Gray* and HN Jabbour) from NHS Lothian Research and Development Office. The project is entitled: " Prokineticin-1 and placental function in normal and pre-eclemptic placentae (£5,300).
6. Programme grant from the Medical Research Council to support my core research within the unit entitled "Prostaglandin signalling and function in uterine physiology and pathology". (April 2006-March 2011; £3,899,119).
7. Project grant (F Denison* and HN Jabbour) from the Moray Endowment Fund entitled "Expression and regulation of prokineticins in normal and pre-eclamptic placentae". (January 2006, £1,890).
8. Project grant (AA Katz, HN Jabbour and RP Millar) from the South African Medical Research Council entitled "The role of cyclooxygenases and prostaglandins in cervical cancer and uterine patholol gy". (January 2007-December 2010, £157,000).
9. Funding from Ardana Biosciences (HN Jabbour) to conduct research on: "Cross talk between prostanoid receptors and GnRH receptors and their relevance to benign hormone dependant pathologies of the endometrium" (October 2005-September 2008, £237,000).
10. Funding from Ardana Biosciences (HN Jabbour) to conduct research in collaboration with the University of Cape Town and Groote Schuur Hospital on: " Elucidation of prostaglandin receptor expression and signalling in endometriosis." This project was

run in collaboration with Professor Arieh Katz and Professor Zephne Van Der Spuy (October 2004-September 2007, £52,000).

11. Funding from Ardana Biosciences (HN Jabbour) to conduct research on development of cDNA arrays analysis of target genes that will assist in investigating the role of prostaglandins and angiogenic genes in endometrial pathologies. The project is run in collaboration with Professor Peter Ghazal at Scottish Centre for Genomic Technology and Informatics (ScGTI) at the University of Edinburgh (March 2005, £50,000).
12. College of Medicine and Veterinary Medicine Fellowship awarded to Dr Oliver Milling-Smith* to conduct a MD degree with Dr HN Jabbour and Professor HOD Critchley*. The tile of the Fellowship was "Investigation of the role of prostaglandin E_2 (PGE_2) signalling pathways in menstrual dysfunction". (September 2003-August 2005).
13. One year Scholarship from the Commonwealth for Ms Melissa Muller to undertake studies on the role of seminal plasma prostaglandins in cervical pathogenesis (2003-2004; £13,000).
14. Project grant (AA Katz, HN Jabbour, CA Flanagan and RP Millar) from the South African Medical Research Council entitled "The role of cyclooxygenase and prostaglandins in neoplastic transformation of epithelial cells of the cervix". (January 2003-December 2006, £44,500).
15. Two year funding from Ardana Biosciences (HN Jabbour) to conduct research investigating the role of PGE_2 in epithelial/endothelial cell function of the human endometrium (November 2001-January 2004, £181,998).
16. Project grant (HN Jabbour and HOD Critchley*) from WellBeing and Royal College of Obstetricians and Gynaecologists entitled "Decidual prolactin and its role in implantation and early pregnancy". (September 2000-August 2002, £73,862).
17. Programme grant from the Medical Research Council to support my core research within the unit entitled "the molecular pathways mediating the role of COX enzymes and prostaglandins in menstruation and epithelial/endothelial cell function and differentiation". (April 2000-March 2005; £1,377,065).
18. Project grant (HN Jabbour) from BBSRC (Biotechnology and Biological Science Research Council) entitled "Seasonal variation in expression and distribution of different prolactin receptor forms in the testis of red deer". (April 1996-March 1998, £60,656).
19. Project grant (HN Jabbour and APF Flint) from The Ministry of Agriculture, Fisheries and Food (MAFF) entitled "Maternal recognition of pregnancy in red deer" (October 1995-September 1998: £72, 500).
20. Project Grant (HN Jabbour and ASI Loudon) from The Ministry of Agriculture, Fisheries and Food (MAFF) entitled "Reproductive technology in deer" (January 1994-December 1996; £138,000).
21. European Community Marie Curie Fellowship entitled "Development of alternative procedures for superovulation and simple techniques for embryo microsurgery and cryopreservation". The fellowship was awarded to Dr Sanos from The University of Philippines at Los Banos under the supervision of HN Jabbour. The fellowship was tenable for one year at The Institute of Zoology in London (February 1994-January 1995; £39, 836).

22. Royal Society short term travel fellowship for an overseas study visit to investigate the expression of a novel clone coding for the prolactin binding protein. The fellowship was tenable at The Molecular Endocrinology laboratory in Faculte de Medecine Necker-Enfants Malades in Paris (October 1994-January 1995; £1,700).
23. PhD studentship from the Agricultural Food Research Council entitled "Physiological function of prolactin based on studies of hormone receptor gene expression" (October 1993-September 1996).
24. Veterinary Research Fellowship from the Agricultural and Food Research Council entitled "The development and application of reproductive technologies in deer" awarded to David Bainbridge under the supervision of HN Jabbour and ASI Loudon (October 1993-September 1996).
25. Wain Travel Award to investigate the *in vitro* expression of a prolactin receptor cDNA clone isolated from red deer liver cDNA library. The award was tenable at The Molecular Endocrinology laboratory in Faculte de Medecine Necker-Enfants Malades in Paris (April 1993).
26. Fellowship from the European Molecular Biology Organisation (EMBO) to work on the cloning and sequencing of the prolactin receptor cDNA in red deer. The fellowship was tenable at the Molecular Endocrinology laboratory in Faculte de Medecine Necker-Enfants Malades in Paris (September 1992).

Research supervision experience:
Total of 17 PhD students (11 as principal supervisor) and 6 MSc students (4 as principal supervisor) supervised. Currently supervising 6 PhD students (2 as principal supervisor).

Teaching experience:
I regularly participate in teaching on the MSc course in reproductive biology and Honours courses in reproductive biology and experimental pathology. I have supervised numerous projects for the University at both MSc and Honours level.

Honours projects supervised:

1. Miss Kimberley Durno (2008). Honours in pharmacology, University of Edinburgh. Thesis Title: "A characterisation of prokineticin expression in the human fallopian tube: Potential regulation of embryo transport".
2. Ms Samantha McDonald (2005). Honours in reproductive Biology, University of Edinburgh Medical School. Thesis title: "An investigation into a possible paracrine regulation of endometrial epithelial cell function by $PGF_{2\alpha}$-FP receptor interaction via FGF2 and ERK1/2 pathways".
3. Miss EmmaJayne Kingham (2005). Honours in pharmacology, University of Edinburgh Medical School. Thesis title: "Thromboxane signalling and receptor regulation in the endometrium".
4. Ms Samantha Gardner (2004). Honours in Pharmacology, University of Edinburgh Medical School. Thesis title: "Seminal plasma prostaglandins and signalling via the EP2 receptor".
5. Miss Suzannah Ackroyd (2001). Honours in Pharmacology, University of Edinburgh Medical School. Thesis title: "Prolactin signalling in the human endometrium".

6. Miss Catherine Bousfield (2001). Honours in Pharmacology, University of Edinburgh Medical School. Thesis title: "Progesterone receptor expression in endometrial cells: effect of prostaglandins". The project was awarded First Class Honours and the Science Prize.
7. Miss Joanne Pegg (1999). Honours in Pharmacology, University of Edinburgh Medical School. Thesis title: "Prolactin receptor in placenta, fetal membranes and decidua in women at term: localisation and functionality". The project was supervised jointly with Dr S Riley. The project was awarded First Class Honours and the SmithKline Beecham Prize.

Postgraduate students supervised:

Ph.D. students:

1. Miss Sharon Eddie (2008-2011) supervised jointly with Professor Richard Anderson and funded by the College of Medicine and Veterinary Medicine of The University Edinburgh. Thesis title: "Local mediators of human germ cell development".
2. Miss Jackie Maybin (2007-2010) supervised jointly with Professor Hilary Critchley and Dr Nik Hirani and funded by the Medical Research Council. Thesis title: "Endometrial repair and regeneration".
3. Mr Jason Sutherland (2007-2010) supervised jointly with Professor Arieh Katz and funded by the MRC Human Reproductive Sciences Unit and The University of Cape Town. Enrolled for a PhD at the University of Cape Town. Thesis title: "The effect of seminal plasma prostaglandins on cervical cancer tumourigenesis".
4. Miss Margaret Keightley (2006-2009) funded by MRC and jointly supervised with Dr Kurt Sales. Thesis tilte: "Prostaglandin receptor regulation of vascular function in reproductive tract cancers".
5. Miss Alison Wallace (2006-2009) funded by MRC and jointly supervised with Dr Adam Pawson. Thesis title: "Ligand induced signalling via the FP receptor".
6. Mr Aron Abera (2005-2008) supervised jointly with Professor Arieh Katz and funded jointly by the MRC Human Reproductive Sciences Unit and The University of Cape Town. Enrolled for a PhD at the University of Cape Town. Thesis title: "Cyclooxygenase and prostanoid receptor expression and signalling in uterine fibroids".
7. Dr Jemma Evans (2004-2008) funded by MRC. Thesis title: "Novel regulators of angiogenesis in the uterus".
8. Dr Oliver Milling-Smith (2003-2006) funded by the College of Medicine and Veterinary Medicine Fellowship to conduct a MD degree with Dr HN Jabbour and Professor Hilary Critchley. Thesis title: "Investigation of the role of prostaglandin E_2 (PGE_2) signalling pathways in menstrual dysfunction".
9. Dr Melissa Muller (2001-2005) funded jointly by the MRC Human Reproductive Sciences Unit and The University of Cape Town. Ms Muller was the recipient of a one year Commonwealth Scholarship tenable at the MRC Human Reproductive Sciences Unit. Enrolled for a PhD at the University of Cape Town. Thesis title: " The molecular pathways mediating the role of cyclooxygenase enzymes and prostaglandins in cervical neoplasias.

10. Dr Kurt Sales (1999-2002) funded jointly by the MRC Human Reproductive Sciences Unit and The University of Cape Town. Enrolled for a PhD at the University of Cape Town. Thesis title: "Expression and functional role of cyclooxygenase enzymes in cervical neoplasias".
11. Dr Gabrielle Perchick (1999-2002) funded by MRC. Thesis title: "Expression and intracellular signalling pathways of PGE receptors in the human endometrium".
12. Dr Annette Dalrymple (1996-2000) funded by MRC. Thesis Title: "Molecular investigations of the role of prolactin on endometrial function during the oestrous cycle and early pregnancy of the common marmoset monkey *Callithrix callithrix*".
13. Dr Mathew Richardson (1995-1999) funded by MRC. Thesis title: "Cellular mechanism of melatonin action in the pituitary gland". The project is jointly supervised with Dr GA Lincoln.
14. Dr Kristina Bird (1995-1999) funded by MAFF. Thesis title: "Maternal recognition of pregnancy in red deer". The project is jointly supervised with Professor APF Flint. Kristina Bird was awarded the Walpole award at the 1999 summer meeting of the Society for the Study of Fertility following a presentation of some of her PhD research data.
15. Dr Luka A Clarke (1993-1997) funded by BBSRC. Thesis title: "Physiological function of prolactin based on studies of hormone receptor gene expression."
16. Dr Dave Thomas (1992-1997) funded by BBSRC. Thesis title: "The hormonal control of hair growth in the red deer". The project was jointly supervised with Professor ASI Loudon.
17. Dr David RJ Bainbridge (1993-1996) funded by BBSRC. Thesis title: "Endocrine regulation of luteal regression and maternal recognition of pregnancy in the red deer (*Cervus elaphus*)". David RJ Bainbridge was awarded the Walpole award at the 1997 summer meeting of the Society for the Study of Fertility following a presentation of some of his PhD research data.

M.Sc. students supervised:

1. Mrs Omaima Idriss (2008). Masters (by research) in Reproductive Biology, University of Edinburgh Medical School. Thesis title: "Regulation of Adrenomedullin expression by $PGF_{2\alpha}$-FP receptor".
2. Mr Omih Edwin (2007). Masters (by research) in Reproductive Biology, University of Edinburgh Medical School. Thesis title: "In vitro modelling of human trophoblast uterine epithelial signalling during implantation using in vitro differentiated hESCs (RH1 and RH4) and immortalised endometrial cell (HES) line".
3. Ms Georgia Papakleovoulou (2004). MSc by Research in Reproductive Biology, University of Edinburgh Medical School. Thesis title: "Signalling pathways activated by membrane progesterone receptors". This project is jointly supervised with Dr Tony Bramley.
4. Mr Oba Harding (2003). MSc research project in Reproductive Biology, University of Edinburgh Medical School. Thesis title "Expression and signalling of the membrane bound progesterone receptor in the human endometrium". This project was jointly supervised with Professor Hilary Critchley and Dr Tony Bramley.

5. Miss Nadine Smith (2000). MSc by Research in Life Sciences, Graduate School of Life Sciences, The University of Edinburgh. Thesis title: "Expression of Cox-1 and Cox-2 enzymes in the primate ovary". This project was jointly supervised with Dr Hamish Fraser.
6. Miss Lucy Twist (1997). MSc in Reproductive Biology, University of Edinburgh Medical School. Thesis title: "Localisation of the prolactin receptor within the sheep ovary and it's role in regulating steroidogenesis by the follicle". The project was jointly supervised with Dr BK Campbell and Professor DT Baird.

Administrative experience:

I have chaired and been a member of numerous committees and councils and been involved in the organisation of several national and international conferences (see list below).

Membership of academic societies:

1. Member of the American Endocrine Society
2. Member of the Society for the Study of Reproduction and Fertility
3. Member of the British Endocrine Society
4. Member of Munro Kerr Society

Membership of committees, etc.:

1. Member of the Editorial Board of Journal of Clinical Endocrinology and Metabolism (a journal of the American Endocrine Society; January 2007-December 2010).
2. Member of the Editorial Board of Recent Patents on Endocrine, Metabolic and Immune Drug Discovery (January 2007 – present).
3. Member of the Editorial Board of Reproduction (a journal of the Society of Reproduction and Fertility; January 2002 -present).
4. Member of the Editorial Board of Journal of Endocrinology (a journal of the British Society for Endocrinology; January 2002 - present).
5. Elected Programme Secretary of the Society of Reproduction and Fertility (2005-2008).
6. Member of the Council of Management of the Society for Reproduction and Fertility (2003-2009).
7. Member of the Organising Committee of the First World Congress of Reproductive Biology held in Hawaii May 24^{th}-26^{th}, 2008.
8. Member of the organising committee of the Simpson Symposium entitled "Cellular communication and signalling in the reproductive tract" held in Edinburgh August 28^{th}-31^{st}, 2005.
9. Regional Representative in Scotland for the British Society of Endocrinology (2001-present).
10. Member of the MRC Human Reproductive Sciences Unit Career Development Fellows Committee. The committee overseas training of postdoctoral scientists and advises on appointments to staff scientist positions (2004-present).
11. Member of the MRC Human Reproductive Sciences Unit computer committee (2004-present).

12. Biological Safety Officer trainer within the MRC Human Reproductive Sciences Unit (2000-present).
13. Member of the MRC-RBU Ethical Review Process: Animal Care and Use Committee (1999-2003).
14. Chair of the Seminar Organising Committee of the Centre for Reproductive Biology (1997-present).
15. Member of the Public Relations and Fund Raising committee at The Institute of Zoology (1994-1995).
16. Chairman of the Seminar Organising Committee at the Institute of Zoology in London (1994-1995).

Invited presentations to international and national meetings:

1. Invited plenary speaker at the 4th Society for Gynaecological Investigation (SGI) endometrial satellite symposium to be held in Glasgow (March 2009). Title: "Prokineticins: Novel regulators of reproductive function".
2. Invited plenary speaker at the Annual Conference of the British Fertility Society held in Liverpool (September 2008). Title: "Local communication in the uterus during implantation".
3. Invited Speaker at the Annual Conference of the Society for Reproduction and Fertility held in Edinburgh (July 2008). Title: "GPCR signalling in health and disease".
4. Invited speaker at the World congress in Reproductive Biology held in Hawaii (May 2008). Title: "Prokineticins: are they novel regulators of implantation and placental function?".
5. Invited speaker at the Medical Research Council Inflammation Road Show held in Edinburgh (September 2007). Title: "Targeting inflammatory signals in the reproductive tract".
6. Invited speaker at the 11th World Congress on Advances in Oncology and 9th International Symposium on Molecular Medicine held in Crete-Greece (October 2006). Title: "Prostaglandin receptors are mediators of vascular dysfunction in endometrial pathologies".
7. Invited speaker at The Centre for Molecular Medicine, University of Connecticut Health Centre (June 2006). Title: "Vascular function in endometrial physiology and pathology".
8. Invited speaker at the 196th Meeting of the British Endocrine Society held in London (November 2005). Title: "Peri-pre-implantation paracrinology".
9. Invited speaker at STADY 2005 conference in Tel Aviv (October 2005): International Symposium on Signal Transduction in Health and Disease. Title: "Prostaglandin receptor function in endometrial pathologies".
10. Invited speaker at the Simpson Symposium held in Edinburgh (August 2005). Title: "Prostaglandin receptors are mediators of vascular function in endometrial pathologies".
11. Invited speaker to the 2nd International Conference on Phospholipases A_2 and 8th International congress on Platelet-Activating factor and Related Lipid Mediators held in Berlin (October 6-9, 2004). Title: "Prostaglandin receptor signalling and function in human endometrial pathology".
12. Invited speaker at a course entitled "Implantation and early development" organized by Royal College of Obstetricians and Gynaecologists (September 20-22, 2004). Title: "Endocrine-paracrine regulation of endometrial function".

13. Invited speaker at 8th International Society for Contraception congress held in Edinburgh (June 4th 2004). Title: "The uterus as a target for contraception and therapeutic intervention".
14. Invited speaker at symposium entitled "Aspirin in the treatment of cancer" organised by the Aspirin Foundation and held at Barts and the London School of Medicine and Dentistry (November 10th, 2003). Title: "COX, prostaglandins and the seminal plasma burden in cervical cancer".
15. Invited speaker on course entitled "New Horizons in Recurrent Pregnancy Loss" organised by Professor Leslie Regan, Imperial College London, Division of Paediatrics, Obstetrics and Gynaecology (September 10-12, 2003). Title: "Systemic and decidual immune responses in early pregnancy".
16. Invited speaker at Workshop funded by the Wellcome Trust and The Royal Society of Edinburgh entitled "Tissue and vascular remodelling in the female reproductive tract" (January 22, 2003; Edinburgh). Title: Second messenger systems in reproductive tract physiology and pathology".
17. Invited speaker at special symposium on maintenance of pregnancy held at the 193rd Meeting of the Society for Endocrinology at the Royal College of Physicians (November 2002; London). Title: Paracrine control of uterine differentiation and implantation.
18. Invited plenary speaker at the International Symposium on Spermatology (October 2002, Cape Town). Title: Cox, prostaglandins and seminal plasma burden on the cervix.
19. Invited speaker at the European Society for Domestic Animal Reproduction held in Vienna (September 2001). Title: Reproductive technology and its potential for conservation of endangered deer species.
20. Invited plenary speaker at The Central European Conference on Animal Reproduction, Poland (September 1996). Title: Reproduction strategies in seasonal wild mammals.
21. Invited speaker at the annual meeting for The Society of British Veterinary Zoology, Edinburgh (March 1995). Title: Manipulation of Reproduction: New approaches to conservation.
22. Invited speaker at a conference organised by the Vietnamese National Centre for Scientific Research held in Hanoi (March 1994). Title: Reproductive technology: a role for preservation of indigenous species in Vietnam.
23. Invited speaker at the Society of Animal Breeding, Linnaean Society in London (November, 1992). Title: Reproductive technology and its application for conservation of endangered ungulate species.
24. Invited speaker at The Second International Symposium on Game Ranching, Edmonton, Canada (June 1990). Title: Controlled breeding and its application for deer.

Invited seminars, etc.:

1. Invited Seminar Speaker at the Centre for Cardiovascular Science (Edinburgh; November 2008). Title: "Lipid signalling in reproductive health and disease".
2. Invited Seminar Speaker at the Royal Veterinary College (London; March 2008). Title: "Targeting inflammatory signals in female reproductive pathology".
3. Invited Speaker at the Centre for Reproductive Biology series of public lectures (March 2008). Title: Periods: a bleeding pain".

4. Invited speaker at The Department of Obstetrics and Gynaecology, School of Medicine, University of Aberdeen (March 2007). Title: "Prostaglandin receptors are regulators of vascular dysfunction in endometrial pathologies".
5. Invited speaker at The Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol (May 2005). Title: "Prostaglandin receptor signalling and function in human endometrial pathology".
6. Invited Speaker at the Institute of Reproductive and Developmental Biology, Imperial College School of Medicine, London (July 2001). Title: "Prolactin signalling and function in the human endometrium".
7. Invited speaker at The Department of Obstetrics and Gynaecology, University of Cambridge (November 2000). Title: "Local regulation of reproductive events: paracrine role for prolactin".
8. Invited Speaker at the Gynaecological Oncology Unit at Groote Schuur Hospital in Cape Town (March 1999). The talk was entitled: "The role of Cox enzymes and prostaglandins in epithelial cell transformation".
9. Invited speaker at the School of Environmental Health at Harvard University (May 1998). The talk was entitled: "Prolactin and uterine function in primates".
10. Invited speaker at the Endocrine Research Group, School of Biological Sciences, The University of Manchester (May 1997). Title: "Prolactin receptor expression: a key regulator of reproductive function".
11. Invited speaker at The Royal Veterinary College (March 1995), The Babraham Institute (September 1995) and The American University of Beirut (August 1997).
12. Invited speaker at the Medical Research Council Reproductive Biology Unit, Edinburgh (March 1994). Title: "Prolactin and circannual rhythms".

External examining for the award of doctoral degrees:

1. Monash University - 2009
2. Imperial College London – 2008
3. University of Manchester – 2006
4. Imperial College – 2006
5. The Royal Veterinary College – 2006
6. The University of Adelaide – 2005
7. University of Cambridge – 2005
8. University of Cambridge - 2004

Other relevant information:

Refereeing:

Referee for peer reviewed scientific journals (Journal of Clinical Endocrinology and Metabolism, Endocrinology, FEBS Letters, British Journal of Pharmacology, Journal of Reproduction and Fertility, Journal of Endocrinology, Journal of Molecular Endocrinology, General and Comparative Endocrinology, Biology of Reproduction, Theriogenology, Reproduction, Fertility and Development) and research grant applications for BBSRC, WellBeing, The Wellcome Trust and the Health Research Council of New Zealand.

Academic awards:

Young Scientist Award, 51st Annual Conference, New Zealand Society of Animal Production 1991

Post-doctoral Fellowship, New Zealand Ministry of Agriculture and Fisheries (1989-1991)

Postgraduate open scholarship from the Australian Wool Corporation (1985-1989)

Scholarship the Agency for International Development (1982-1983)

Graduated with Distinction (1982)

Munzer Wehbeh Scholarship (1981-1982)

Patents (University of Edinburgh collaborators marked by *):

1. ARDW/P25288GB and ARDW/P25288US – The application of EP2 and EP4 receptor antagonists in the treatment of menorrhagia. Inventors: HN Jabbour and HOD Critchley*.
2. ARDW/P26373GB and ARDW/P28269PC – The application of FP receptor antagonist in the treatment of menorrhagia. Inventors: HN Jabbour and HOD Critchley*.
3. ARDW/P25932GB and ARDW/P28268PC – The application of FP receptor antagonist in the treatment of uterine disorders. Inventors: HN Jabbour, S Milne and HOD Critchley*.
4. ARDW/P27931GB – The application of PGI antagonist in the treatment of menorrhagia. Inventors: HN Jabbour and HOD Critchley*.
5. ARDW/P28446GB – The application of PGI receptor antagonist in the treatment of menorrhagia. Inventors: HN Jabbour and Hilary Critchley*.
6. ARDW/P28312GB – The antagonism of prokineticin receptors in the treatment of menorrhagia. Inventors: HN Jabbour, RP Millar and HOD Critchley*.
7. 60/340971US – The application of COX enzyme inhibitors and EP receptor antagonists in the treatment of cervical cancer. Inventors: HN Jabbour, KJ Sales and A Katz.
8. 60/333562US – The application of COX inhibitors and EP receptor antagonists in the treatment of uterine cancer. Inventor: HN Jabbour.
9. The application of combination treatment of GnRH analogue with an inhibitor of prostaglandin synthesis or prostaglandin receptor antagonist in the treatment of sex-hormone dependant disease and fertility treatment. Inventors: Z Naor, HN Jabbour and RP Millar.
10. The application of phosphodiesterases in menorrhagia. Inventors: HN Jabbour, O Milling-Smith and HOD Critchley*.

Publications:

PhD Thesis

Jabbour, H.N. (1989). Studies on fertility of superovulated ewes. The University of Sydney.

Research papers in refereed scientific journals:

1. Evans, J., Catalano, R.D., Brown, P., Sherwin, R., Critchley, H.O.D., Fazleabas, A.R. and Jabbour, H.N. (2009). Prokineticin 1 mediates fetal-maternal dialogue regulating endometrial leukaemia inhibitory factor. **FASEB Journal** (in press).

2. **Evans, J., Catalano, R., Morgan, K., Critchley, H.O.D., Millar, R.P. and Jabbour, H.N. (2008). Prokineticin 1 signalling and gene regulation in early human pregnancy. **Endocrinology** 149: 2877-2887.

3. Denison, F.C., Battersby, S., King, A.E., Szuber, M. and Jabbour, H.N. (2008). Prokineticin 1 - a novel mediator of the inflammatory response in third trimester human placenta. **Endocrinology** 149: 3470-3477.

4. **Sales, K.J., Boddy, S.C. and Jabbour, H.N. (2008). F-prostanoid receptor alters adhesion, morphology and migration of endometrial adenocarcinoma cells. **Oncogene** 27: 2466-2477.

5. Sales, K.J., Grant, V. and Jabbour, H.N. (2008). Prostaglandin E_2 and $F_{2\alpha}$ activate the FP receptor and up-regulate cyclooxygenase 2 expression via the cAMP response element. **Molecular and Cellular Endocrinology** 285: 51-61.

6. Battersby, S., Sales, K.J., Williams, A.R., Anderson, R.A., Gardner, S. and Jabbour, H.N. (2007). Seminal plasma and PGE_2 up-regulate fibroblast growth factor-2 expression in endometrial adenocarcinoma cells via E-series prostanoid-2 receptor-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase pathway. **Human Reproduction** 22: 36-44.

7. Milling Smith O.P., Jabbour, H.N. and Critchley, H.O.D. (2007). Cyclooxygenase enzyme expression and E-series prostaglandin receptor signalling are enhanced in heavy menstruation. **Human Reproduction** 22: 1450-1456.

8. Naor, Z., Jabbour, H.N., Naidich, M., Pawson, A.J., Morgan, K., Battersby, S., MacPherson, S., Millar, M., Brown, P. and Millar, R.P. (2007). Reciprocal cross-talk between Gonadotrophin-releasing Hormone (GnRH) and prostaglandin receptors regulates GnRH receptor expression and differential gonadotrophin secretion. **Molecular Endocrinology** 21:524-537.

9. Sales, K.J., Boddy, S.C., Williams. A.R.W., Anderson, R.A. and Jabbour, H.N. (2007). F-prostanoid regulation of fibroblast growth factor 2 signalling in endometrial adenocarcinoma cells. **Endocrinology** 148: 3635-3644.

10. Qualtrough, D., Kaidi, A., Chell, S., Jabbour, H.N., Williams, A. and Paraskeva, C. (2007). Prstaglandin $F_{2\alpha}$ stimulates migration and invasion in colorectal tumour cells. **International Journal of Cancer** 121: 734-740.

11. Critchley, H.O.D., Osei, J., Henderson, T.A., Boswell, L., Sales, K.J., Jabbour, H.N. and Hirani, N. (2006). Hypoxia-inducible factor-1α expression in human endometrium and its regulation by prostaglandin E-series prostanoid receptor 2 (EP2). **Endocrinology** 147: 744-753.

12. Milling Smith O.P., Battersby, S., Sales, K.J., Critchley, H.O.D. and Jabbour, H.N. (2006). Prostacyclin receptor up-regulates endometrial expression of angiogenic genes in human endometrium via cross talk with epidermal growth factor receptor and the extracellular signalling receptor kinase 1/2 pathway. **Endocrinology** 147: 1697-1705.

13. Muller, M., Sales, K.J., Katz, A.A. and Jabbour, H.N. (2006). Seminal plasma promotes the expression of tumorigenic and angiogenic genes in cervical adenocarcinoma cells via the E-series prostanoid 4 receptor. **Endocrinology** 147: 3356-3365.

14. **Jabbour, H.N., Sales, K.J., Boddy, S.C., Anderson, R.A. and Williams, A.R.W. (2005). A positive feedback loop that regulates cyclooxygenase-2 expression and prostaglandin $F_{2\alpha}$ synthesis via the F-series-prostanoid receptor and extracellular signal-regulated kinase 1/2 signalling pathway. **Endocrinology** 146:4657-4664.

15. **Sales, K.J., List, T., Boddy, S.C., Williams, A.R.W., Anderson, R.A., Naor, Z. and Jabbour, H.N. (2005). A novel angiogenic role for prostaglandin $F_{2\alpha}$-FP receptor interaction in human endometrial adenocarcinoma. **Cancer Research** 65: 7707-7716.

16. Battersby, S., Critchley, H.O.D., de Brum-Fernandes, A.J. and Jabbour, H.N. (2004). Temporal expression, localisation and signalling of prostacyclin receptor in the human endometrium across the menstrual cycle. **Reproduction** 127: 79-86.

17. Battersby, S., Critchley, H.O.D., Morgan, K., Millar, R.P. and Jabbour, H.N. (2004). Expression and regulation of prokineticins (EG-VEGF and Bv8) and their receptors in the human endometrium across the menstrual cycle. **Journal of Clinical Endocrinology and Metabolism** 89: 2463-2469.

18. **Sales, K.J., Milne. S.A., Williams, A.R.W., Anderson, R.A. and Jabbour, H.N. (2004). Expression, localisation and signalling of Prostaglandin $F_{2\alpha}$ receptor in human endometrial adenocarcinoma: regulation of proliferation by activation of the Epidermal Growth Factor Receptor and Mitogen Activated Protein kinase signalling pathways. **Journal of Clinical Endocrinology and Metabolism** 89: 986-993.

19. **Sales, K.J., Maudsley S. and Jabbour, H.N. (2004). Elevated prostaglandin EP2 receptor in endometrial adenocarcinoma cells promotes VEGF expression via cAMP-meidated transactivation of the EGF receptor and ERK1/2 pathways. **Molecular Endocrinology** 18: 1533-1545.

20. Sales, K.J., Battersby, S., Williams, A.R.W., Anderson, R.A. and Jabbour, H.N. (2004). Prostaglandin E_2 mediates phosphorylation and down-regulation of the tuberous sclerosis-2 tumour suppressor (tuberin) in human endometrial adenocarcinoma cells via the Akt signalling pathway. **Journal of Clinical Endocrinology and Metabolism** 89: 6112-6118.

21. Jabbour, HN and Boddy, S.C. (2003). Prostaglandin E_2 induces proliferation in glandular epithelial cells of the human endometrium via ERK mediated pathway. **Journal of Clinical Endocrinology and Metabolism** 88: 4481-4487.

22. **Milne, S.A. and Jabbour H.N. (2003). Prostaglandin $F_{2\alpha}$ receptor expression and signalling in human endometrium: role of $PGF_{2\alpha}$ in epithelial cell proliferation. **Journal of Clinical Endocrinology and Metabolism** 88: 1825-1832.

23. Perchick, G.B. and Jabbour, H.N. (2003). COX-2 over-expression inhibits Cathepsin-D mediated cleavage of plasminogen to the potent anti-angiogenic factor angiostatin. **Endocrinology** 144: 5322-5328.

24. Battersby, S., Boddy, S.C., Critchley, H.O.D. and Jabbour, H.N. (2002). Expression and localisation of endothelial monocyte-activating peptide II in the human endometrium across the menstrual cycle: Regulation of expression by prostaglandin E_2. **Journal of Clinical Endocrinology and Metabolism** 87: 3928-3935.

25. **Gubbay, O., Critchley, H.O.D., Bowen, J.M., King, A. and Jabbour H.N. (2002). Prolactin induces ERK phosphorylation in epithelial and $CD56^+$ natural killer cells of the human endmetrium. **Journal of Clinical Endocrinology and Metabolism** 87: 2329-2335.

26. Hair, W.M., Gubbay, O., Jabbour, H.N. and Lincoln, G.A. (2002). Prolactin receptor expression in human testis and accessory tissues: localisation and function. **Molecular Human Reproduction** 8: 606-611.

27. **Sales, K.J., Katz, A.A., Howard, B., Soeters, R.P, Millar, R.P. and Jabbour, H.N. (2002). Cyclooxygenase-1 is up-regulated in cervical carcinomas: Autocrine/paracrine regulation of cyclooxygenase-2, PGE receptors and angiogenic factors by cyclooxygenase-1. **Cancer Research** 62: 424-432.

28. **Sales, K.J., katz, A.A., Millar, R.P. and Jabbour, H.N. (2002). Seminal plasma activates cyclooxygenase-2 and prostaglandin E_2 receptor expression and signalling in cervical adenocarcinoma cells. **Molecular Human Reproduction** 8: 1065-1070.

29. Jabbour, H.N., Kelly, R.W. and Boddy, S.C. (2002). Autocrine/paracrine regulation of apoptosis in epithelial cells by prostaglandin E_2. **Prostaglandins, Leukotreines and Essential Fatty Acids** 67: 357-363.

30. **Jabbour, HN, Milne, S.A., Williams, A.R., Anderson, R.A. and Boddy, S.C. (2001). Expression of COX-2 and PGE synthase and synthesis of PGE_2 in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. **British Journal of Cancer** 85: 1023-1031.

31. Lincoln, G.A., Townsend, J. and Jabbour, HN (2001). Prolactin actions in the sheep testis: a test of the priming hypothesis. **Biology of Reproduction** 65: 936-943.

32. **Milne, S.A., Perchick, G., Boddy, S.C. and Jabbour, H.N. (2001). Expression, localisation and signalling of PGE_2 and EP2/EP4 receptors in human non-pregnant endmetrium across the menstrual cycle. **Journal of Clinical Endocrinology and Metabolism** 86: 4453-4459.

33. **Sales, K.J, Katz, A.A., Davies, M., Hinz. S., Soeters, R.P., Hofmeyr, M.D., Millar, R.P. and Jabbour, HN (2001). Cyclooxygenase-2 expression and prostaglandin E_2 synthesis are up-regulated in carcinomas of the cervix: A possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. **Journal of Clinical Endocrinology and Metabolism** 86: 2243-2249.

34. Dalrymple, A. and Jabbour H.N. (2000). Localisation and signalling of the prolactin receptor in the uterus of the common marmoset monkey. **Journal of Clinical Endocrinology and Metabolism** 85: 1711-1718.

35. Dalrymple. A., Edery, M. and Jabbour, H.N. (2000). Sequencing and functional characterisation of the marmoset monkey (*Callithrix jacchus*) prolactin receptor: comparative homology with the human long-form prolactin receptor. **Molecular and Cellular Endocrinology** 167: 89-97.

36. Demmers, K.J., Jabbour, H.N., Deakin, D.W. and Flint, A.P.F. (2000). Production of interferon by red deer (*Cervus elaphus*) conceptuses and the effects of roIFN-τ on the timing of luteolysis and the success of asynchronous embryo transfer. **Journal of Reproduction and Fertility** 118: 387-395.

37. Bainbridge, D.R.J., Evans, G., Catt, S and Jabbour, H.N. (1999). Successful *in vitro* fertilisation of *in vivo* matured oocytes aspirated laparoscopically from red deer hinds *(Cervus elaphus*). **Theriogenology** 51: 891-898.

38. Bainbridge, D.R.J. and Jabbour, H.N. (1999). Source and site of action of antiluteolytic interferon in red deer (*Cervus elaphus*): Possible involvement of extra-ovarian oxytocin secretion in maternal recognition of pregnancy. **Journal of Reproduction and Fertility** 116: 305-313.

39. Demmers, K.J., Kaluz, S., Deakin, D.W., Jabbour, H.N. and Flint, A.P.F. (1999). Production of interferon by the conceptus in red deer *Cervus elaphus*. **Journal of Reproduction and Fertility** 115: 59-65.

40. Jabbour, H.N. and Lincoln GA. (1999). Prolactin receptor expression in the testis of the ram: localisation, functional activation and the influence of gonadotrophins. **Molecular and Cellular Endocrinology** 148: 151-161.

41. Jabbour, H.N., Critchley, H.O.D., Yu-Lee L.Y. and Boddy, S.C. (1999). Localisation of Interferon Regulatory Factor-1 (IRF-1) in the non- pregnant human endometrium: Expression of IRF-1 is up-regulated by prolactin during the secretary phase. **Journal of Clinical Endocrinology and Metabolism** 84: 4260-4265.

42. Bainbridge, D.R.J., Deakin D. and Jabbour, H.N. (1998). Premature luteal regression induced by eCG and estrogen is suppressed by administration of exogenous interferon in red deer (*Cervus elaphus*). **Biology of Reproduction** 58: 124-129.

43. Jabbour, H.N., Clarke, L.A., McNeilly, A.S., Edery, M. and Kelly, P.A. (1998). Is prolactin a gonadotrophic hormone in red deer (*Cervus elaphus*)? Pattern of expression of the prolactin receptor gene in the testis and epididymis. **Journal of Molecular Endocrinology** 20: 175-182.

44. Jabbour, H.N., Clarke, L.A., Bramely, T., Postel-Vinay, M.C., Kelly, P.A. and Edery, M. (1998). Alternative splicing of the prolactin receptor gene generates a 1.7 kb RNA transcript that is linked to prolactin function in the red deer testis. **Journal of Molecular Endocrinology** 21: 51-59.

45. **Jabbour, H.N., Critchley, H.O.D. and Boddy, S.C. (1998). Expression of functional prolactin receptors in nonpregnant human endometrium: Janus Kinase-2, Signal Transducer and Activator of Transcription-1 (STAT1), and STAT5 proteins are phosphorylated after stimulation with prolactin. **Journal of Clinical Endocrinology and Metabolism** 83: 2545-2553.

46. **Jones, R.L., Critchley, H.O.D., Brooks, J., Jabbour, H.N. and McNeilly, A.S. (1998). Localisation and temporal expression of prolactin receptor in human endometrium. **Journal of Clinical Endocrinology and Metabolism** 83: 258-262.

47. Clarke, L.A., Wathes, D.C. and Jabbour, H.N. (1997). Expression and localisation of prolactin receptor messenger ribonucleic acid in red deer ovary during the estrus cycle and pregnancy. **Biology of Reproduction** 57: 865-872.

48. Bainbridge, D.R.J and Jabbour, H.N. (1998). Effect of pregnancy and exogenous interferon on synchronous pulsatile release of oxytocin and luteolytic prostaglandin $F_{2\alpha}$ in red deer (Cervus elaphus). **Journal of Reproduction and Fertility** 111: 299-307.

49. Flint, A.P.F., Albon, S.D., Loudon, A.S.I. and Jabbour, H.N. (1997). Behavioural dominance and corpus luteum function in red deer (*Cervus elaphus*). **Hormones and Behaviour** 31: 296-304.

50. Jabbour, H.N., Hayssen, V. and Bruford, M.W. (1997). Conservation of deer: Contributions from molecular biology, evolutionary ecology, and reproductive physiology. **Journal of Zoology** (London) 243: 461-484.

51. Jabbour, H.N., Boddy, S.C. and Lincoln, G.A. (1997). Pattern and localisation of expression of VEGF and its receptor *flt-1* in the ovine pituitary gland: expression is independent of hypothalamic control. **Molecular and Cellular Endocrinology** 134: 91-100.

52. Bainbridge, D.R.J., Hunter, M.G. Chapple, D.G., Flint, A.P.F. and Jabbour, H.N. (1996). Role of estrogen and prostaglandin $F_{2\alpha}$ in premature luteal regression in monovulatory and superovulated red deer (*Cervus elaphus*). **Biology of Reproduction** 54: 347-354.

53. Bainbridge, D.R.J., Davies, M., Scaramuzzi, R.J. and Jabbour, H.N. (1996). Exogenous interferon delays luteal regression in red deer hinds *(Cervus Elaphus)* by suppressing steroid-induced endometrial oxytocin sensitivity. **Biology of Reproduction** 55: 883-888.

54. Holt, W.V., Abaigar, T. and Jabbour, H.N. (1996). Oestrous synchronisation, semen preservation and artificial insemination in the Mohor gazelle (*Gazella dama mhorr*) for the establishment of a genome resource bank programme. **Reproduction, Fertility and Development** 8: 1215-1222.

55. Jabbour, H.N. and Bainbridge, D.R.J. (1996). Deer: Reproduction and conservation. **Reproduction in Domestic Animal** 31: 501-504.

56. Jabbour, H.N., Clarke, L.A., Boddy, S., Pezet, A., Edery, M. and Kelly, P.A. (1996). Cloning, sequencing and functional analysis of a truncated cDNA encoding red deer prolactin receptor: an alternative tyrosine residue mediates β-casein promoter activation. **Molecular and Cellular Endocrinology** 123: 17-26.

57. Asher, G.W., Jabbour, H.N., Thompson, J.G.E., Tervit, H.R. and Morrow, C.J. (1995). Superovulation of farmed red deer *(Cervus elaphus)* and fallow deer *(Dama dama)*: Incidence of ovulation and changes in plasma hormone concentrations during the preovulatory period in relation to ova recovery and fertilisation. **Animal Reproduction Science** 38: 137-154.

58. Bainbridge, D.R.J., Chapple, D., Loudon, A.S.I. and Jabbour, H.N. (1995). Immunoneutralisation with a monoclonal antibody attenuates the superstimulatory effects of PMSG on the ovarian and endocrine responses in red deer (*Cervus elaphus)*. **Theriogenology** 43: 1339-1350.

59. Clarke, L.A., Edery, M., Loudon, A.S.I., Randall, V.A., Postel-Vinay, M-C, Kelly, P.A. and Jabbour, H.N. (1995). Expression of the prolactin receptor gene during the breeding and non-breeding seasons in red deer *(Cervus elaphus)*: evidence for the expression of two forms. **Journal of Endocrinology** 146: 313-321.

60. Argo, C. McG, Jabbour, H.N., Goddard, P.J., Webb, R. and Loudon, A.S.I. (1994). Superovulation in red deer *(cervus elaphus)* and Pere David's deer *(Elaphurus davidianus)* and fertilisation rates following artificial insemination with Pere David's deer semen. **Journal of Reproduction and Fertility** 100: 629-636.

61. Flint, A.P.F., Jabbour, H.N. and Loudon, A.S.I. (1994). Oxytocin stimulates uterine prostaglandin $F_{2\alpha}$ secretion in red deer *Cervus elaphus*. **Reproduction, Fertility and Development** 6: 269-271.

62. Jabbour, H.N., Veldhuizen, F.A., Mulley, R.A. and Asher, G.W. (1994). Effect of exogenous gonadotrophin on oestrus, the luteinizing hormone surge and the timing and rate of ovulation in red deer *(Cervus elaphus)*. **Journal of Reproduction and Fertility** 100: 533-539.

63. Jabbour, H.N., Marshall, V.S., Argo C. McG., Hooton, J and Loudon, A.S.I. (1994). Successful embryo transfer following artificial insemination of superovulated fallow deer *(Dama dama)*. **Reproduction, Fertility and Development** 6: 181-185.

64. Asher, G.W., Fisher, M.W., Fennessy, P.F., Mackintosh, C.G., Jabbour, H.N. and Morrow, C.J. (1993). Oestrous synchronisation, semen collection and artificial insemination of farmed red deer *(Cervus elaphus)* and fallow deer *(Dama dama)*. **Animal Reproduction Science** 33: 243-267.

65. Jabbour, H.N., Argo, C.McG., Brinklow, B.R., Loudon, A.S.I. and Hooton, J. (1993). Conception rates following intrauterine insemination of European *(Dama dama dama)* fallow deer does with fresh or frozen-thawed Mesopotamian *(Dama dama mesopotamica)* fallow deer spermatozoa. **Journal of Zoology (London)** 230: 379-384.

66. Jabbour, H.N., Veldhuizen, F.A., Green, G. and Asher, G.W. (1993). Endocrine responses and conception rates in fallow deer *(Dama dama)* following oestrous synchronisation and cervical insemination with fresh or frozen-thawed spermatozoa. **Journal of Reproduction and Fertility** 98: 495-502.

67. Asher, G.W., Fisher, M.W., Jabbour, H.N., Smith, J.F., Mulley, R.C., Morrow, C.J., Veldhuizen, F.A. and Langridge, M. (1992). Temporal relationship between the onset of oestrus, the preovulatory LH surge and ovulation following oestrous synchronisation and superovulation of farmed red deer, *Cervus elaphus*. **Journal of Reproduction and Fertility** 96: 261-273.

68. Asher, G.W., Morrow, C.J., Jabbour, H.N., Mulley, R.C., Veldhuizen, F.A. and Langridge, M. (1992). Laparoscopic intrauterine insemination of fallow deer with frozen-thawed or fresh semen. **New Zealand Veterinary Journal** 40: 8-14.

69. Jabbour, H.N., Asher, G.W., Smith, J.F. and Morrow, C.J. (1992). Effect of progesterone and oestradiol benzoate on oestrous behaviour and secretion of luteinizing hormone in ovariectomized fallow deer *(Dama dama)*. **Journal of Reproduction and Fertility** 94: 351-359.

70. Jabbour, H.N. and Evans, G. (1991). Fertility of superovulated ewes following intrauterine or oviductal insemination with fresh or frozen-thawed semen. **Reproduction, Fertility and Development** 3: 1-17.

71. Jabbour, H.N. and Evans, G. (1991). Ovarian and endocrine responses of Merino ewes following treatment with PMSG and GnRH or PMSG antiserum. **Animal Reproduction Science** 24: 259-270.

72. Jabbour, H.N. and Evans, G. (1991). The ovarian and endocrine responses of Merino ewes to treatment with PMSG and/or FSH-P. **Animal Reproduction Science** 26: 93-106.

73. Jabbour, H.N. and Evans, G. (1991). Superovulation of Merino ewes with an ovine pituitary FSH extract. **Reproduction, Fertility and Development** 3: 561-569.

74. Jabbour, H.N., Ryan, J.P. Evans, G. and Maxwell, W.M.C. (1991). The production of viable sheep embryos for embryo transfer programmes. 3. Effects of season, GnRH administration and lupin supplementation on the ovarian and endocrine responses of Merino ewes treated with PMSG and FSH-P to induce superovulation. **Reproduction, Fertility and Development** 3: 699-707.

75. Asher, G.W., Fisher, M.W., Smith, J.F., Jabbour, H.N. and Morrow, C.J. (1990). Temporal relationship between the onset of oestrus, the pre-ovulatory LH surge and ovulation in farmed fallow deer *(Dama dama)*. **Journal of Reproduction and Fertility** 89: 761-767.

76. Pollard, I., Jabbour, H.N. and Mehrabani, P. A. (1987). Effects of caffeine administered during pregnancy on the foetal development and subsequent function in the adult rat: Prolonged effects on a second generation. **Journal of Toxicology and Environmental Health** 22: 1-15.

Invited and refereed review articles in scientific journals:

1. **Maldonado-Perez, D., Evans, J., Denison, F., Millar, R.P., and Jabbour, H.N. (2007). Potential roles of prokineticins in reproduction. **Trends in Endocrinology and Metabolism** 18: 66-72.

2. **Jabbour, H.N., Kelly, R.W., Fraser, H.M. and Critchley, H.O.D (2006). Endocrine regulation of endometrial function. **Endocrine Reviews** 27: 17-46.

3. Jabbour, H.N., Sales, K.J., Milling-Smith, O., Battersby, S. and Boddy, S.C. (2006). Prostaglandin receptors are mediators of vascular function in endometrial pathologies. **Molecular and Cellular Endocrinology** 252: 191-200.

4. Jabbour, H.N. and Sales, K.J. (2004). Prostaglandin receptor signalling and function in human endometrial pathology. **Trends in Endocrinology and Metabolism** 15: 398-404.

5. Sales, K.J. and Jabbour, H.N. (2003). COX enzymes and prostaglandins in reproductive tract physiology and pathology. **Prostaglandins and other Lipid Mediators** 71: 97-217.

6. Sales, K.J. and Jabbour, H.N. (2003). Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. **Reproduction** 126: 559-567.

7. Jabbour, H.N., Gubbay, O. and Critchley, H.O.D. (2002). Prolactin action and signalling in the human endometrium. **Reproductive Medicine Review** 10: 117--132.

8. Jabbour, H.N. and Critchley, H.O.D. (2001). Potential role of decidual prolactin in early pregnancy. **Reproduction** 121: 197-205.

9. Bainbridge, D.R.J. and Jabbour, H.N. (1998). The potential of assisted breeding techniques for conservation of endangered mammalian species: A review. **Veterinary Records** 143: 159-168.

10. Jabbour, H.N. and Kelly, P.A. (1997). Prolactin receptor subtypes: A possible mode of tissue specific regulation of prolactin function. **Reviews of Reproduction** 2: 14-18.

Invited refereed articles in books and conference proceedings:

1. Jabbour, H.N., Sales, K.J. and Critchley, H.O.D. (2005). Endocrine and paracrine signalling in the human endometrium: Potential role for the prostanoid family in implantation. **In: Implantation and early development**. Editors: HOD Critchley, I Cameron and SK Smith, Royal College of Obstetricians and Gynaecologists Press: 3-15.

2. Jabbour, H.N. and Sales, K.J. (2002). Cyclooxygenases in reproductive tract carcinomas: Review". **Scientific Research Flash in Molecular Endocrinology** 1: 1-13.

3. Jabbour, H.N., Sales, K.J., Soeters, R.P., Katz, A.A. and Millar, R.P. (2002). COX, prostaglandins, and seminal plasma burden on the neoplastic cervix. **Proceedings of the 9th International Symposium on Spermatology** (Cape Town, South Africa; Editors: G. Van Der Horst, D. Franken, R. Bornman, T. De Jager and S. Dyer), Monduzzi Editori (Bologna): 217-222.

4. Loudon, A.S.I. and Jabbour, H.N. (1994). Seasonal rhythms in the physiology of deer: endogenous clocks and target tissues. **Recent Development in Deer Biology** (Ed: J.A. Milne): 197-207.

5. Jabbour, H.N. (1992). Reproductive technology in red *(Cervus elaphus)* and fallow *(Dama dama)* deer. **Proceedings of the British Veterinary Deer Society** 5: 3-12.

6. Morrow, C.J., Asher, G.W., Smith, J.F., Jabbour, H.N., Mulley, R.C. and McLeay, L.M. (1992). Seasonal effects on the efficacy of intravaginal CIDR devices for

oestrous synchronisation of farmed fallow deer *(Dama dama)*. **Proceedings of the New Zealand Society of Animal Production** 53: 165-169.

7. Jabbour, H.N. and Asher, G.W. (1991). Artificial breeding of farmed fallow deer *(Dama dama)*. **In: Wildlife Production: Conservation and Sustainable Development** (Editors: L.A. Renecker and R.J. Hudson), pp. 485-491. AFES Misc. Publ. 91-6 (Alaska).

8. Asher, G.W., Jabbour, H.N., Berg, D., Fisher, M.W., Fennessy, P.F. and Morrow, C.J. (1991). Artificial insemination, embryo transfer and gamete manipulation of farmed Red deer and Fallow deer. **Proceedings of a deer course for veterinarians,** Course No. 8; Deer Branch, N.Z. Veterinary Association, Sydney 275-306.

9. Jabbour, H.N. and Asher, G.W. (1991). Techniques of oestrous synchronisation and artificial insemination of farmed fallow deer and red deer. **Proceedings of a Ruakura Farmers Conference;** Ruakura Agricultural Centre, Hamilton, New Zealand, 111-118.

10. Jabbour, H.N., Veldhuizen, F.A., Green, G., Langridge, M. and Asher, G.W. (1991). Fertility of fallow deer *(Dama dama)* does following synchronisation of oestrus with CIDR devices or prostaglandin. **Proceedings of the New Zealand Society of Animal Production** 51: 147-151.

11. Asher, G.W., Jabbour, H.N., Morrow, C.J. and Langridge, M. (1990). Reproductive physiology and controlled breeding of farmed fallow deer. **Proceedings of a deer course for veterinarians,** Course No. 7; Deer Branch, N.Z. Veterinary Association, Auckland: 93-115.

12. Asher, G.W., Jabbour, H.N., Morrow, C.J. and Veldhuizen, F. (1990). Advanced reproduction techniques for farmed fallow deer. **Proceedings of the Fallow Deer Forum '90;** N.Z. Fallow Deer Society, Hawkes Bay: 1-8.

13. Jabbour, H.N., Asher, G.W. and Veldhuizen, F.A. (1990). Semen technology for farmed fallow deer. **Proceedings of the Fallow Deer Forum '90;** N.Z. Fallow Deer Society, Hawkes Bay: 9-13.

Invited articles in the popular press:

1. Jabbour, H.N. (Spring 2005). Prolactin and angiogenesis. **The Endocrinologist** (The newsletter of the Society for Endocrinology).

2. Jabbour, H.N. and Bainbridge, D.R.J. (1997). Endangered deer: the key to their successful conservation. **The Biologist** 44 (1) 253-256.

Research papers under review at refereed journals:

1. Collins, F., MacPherson, S., Brown, P., Bombail, V., Williams, A.R.W., Anderson, R.A., Jabbour, H.N. and Saunders, P.T.K. (2009). Differential expression of ERα and ERβ variants in endometrial cancers and a role for prostaglandins in modulation of ERα and PR. **European Journal of Cancer** (in submission).

2. Maldonado-Perez, D., Brown, P., Morgan, K., Millar, R.P., Thompson, E.A and Jabbour, H.N. (2009). Prokineticin 1 modulates IL-8 expression via the calcineurin/NFAT signalling pathway. **Biophysica Biochemica Acta** (in review).

3. Sales, K.J., Maldonado-Perez, D., Grant, V., Catalano, R.D., Wilson, M.R., Brown, P., Williams, A.R.W., Anderson, R.A., Thompson, E.A. and Jabbour, H.N. (2009). F-Prostanoid receptor regulates proliferation of endometrial adenocarcinoma via the Calcium-Calcineurin-NFAT induction of CXCL8. **Cancer Research** (in submission).

4. Waclawik, A., Jabbour, H.N., Blitek, A. and Ziecik, A.J. (2009). Estradiol-17β, PGE_2 and PTGER2 are involved in PGE_2 positive feedback loop in the porcine endometrium. **Endocrinology** (in submission).

5. Wallace, A.E., Sales, K.J., Catalano, R.D., Anderson, R.A., Williams, A.R.W., Schwarze, J., Wang, H., Rossi, A.G. and Jabbour, H.N. (2009). Prostaglandin F2α-FP signalling promotes neutrophil chemotaxis via CXCL1 in endometrial adenocarcinoma. **Cancer Research** (in submission).

6. Maldonado-Perez, D., Denison, F.C., Jabbour, H.N. and Norman, J.E. (2009). Role of lipoxins as modulators of inflammation during labour in human myometrium. **Journal of Clinical Endocrinology and Metabolism** (in submission).

Annex 2: Chollet et al., "Tocolytic Effect of a Selective FP receptor Antagonist in Rodent Models Reveals an Innovative Approach to the Treatment of Preterm Labor," BMC Pregnancy and Childbirth 7(Suppl 1):S16 (2007).

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Tocolytic effect of a selective FP receptor antagonist in rodent models reveals an innovative approach to the treatment of preterm labor

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Abstract

Background: Management of preterm labor by tocolysis remains an unmet medical need. Prostaglandins play a major role in regulation of uterine activity and in molecular mechanisms of human labor and parturition. There is some circumstantial evidence that prostaglandin F2α by action through the prostaglandin receptor subtype FP is effective in key events during labor uterine contraction, rupture of membranes and cervical dilation. This role of FP is briefly reviewed. In this study, we tested the hypothesis that an orally active and selective FP antagonist may arrest labor and delay parturition in animal models.

Methods: We examined the effects of a small molecule selective antagonist of the FP receptor (AS604872) in inhibition of spontaneous uterine contraction in pregnant rat near term. We tested AS604872 for its ability to delay preterm birth in a mouse model in which the anti-progestin agent RU486 triggered parturition.

Results: By oral or intravenous dosing AS604872 reduced markedly and dose-dependently the spontaneous uterine contractions in late-term pregnant rats at gestational days 19–21. In pregnant mice, AS604872 delayed the preterm birth caused by RU486 administration. The effect was dose-dependent with a significant increase in the mean delivery time of 16 and 33 hours at oral doses of 30 mg/kg and 100 mg/kg, respectively, in the case of labor triggered at gestational day 14. In both models AS604872 appeared more effective than the β-agonist ritodrine.

Conclusion: The tocolytic activity displayed by a selective FP receptor antagonist supports a key role for the FP receptor in the pathophysiology of premature birth and demonstrates the therapeutic potential of an FP antagonist for the treatment of preterm labor cases in which uterine hyperactivity plays a dominant role.

Background

Preterm birth is a serious health problem that remains the major cause of perinatal mortality and morbidity [1]. Spontaneous preterm labor is the major cause of premature birth. Despite important advances in our understanding of the mechanism of human parturition and the pathophysiology of preterm labor, there has been very little progress in the prediction, prevention and therapeutic management of preterm labor. Preterm increase of uterine activity is the most common expression of preterm labor. Therefore, pharmacological interventions aimed at maintaining uterine quiescence have been given much attention for the pharmaceutical management of preterm labor [2]. Tocolytic agents arrest preterm labor and prolong pregnancy up to a few days at the most but often with adverse effects on women and without clear demonstration of improvement in neonatal outcome [3,4].

Prostaglandins play a major role during pregnancy and parturition in all studied species, including human. In particular prostaglandins are involved, directly or through modulation of other endocrine or paracrine factors, in the final common pathway of preparation, activation and stimulation of uterine tissues leading to onset of labour [5-9]. Levels of prostaglandins in uterine tissues vary in time and regionally under the control of their synthesis by cyclooxygenases isozymes COX-1 and COX-2 and specific prostaglandin synthases on one hand and their metabolism by prostaglandin dehydrogenase enzymes on the other hand. Prostaglandins exert their effects through at least nine G protein-coupled receptor subtypes EP1-4, IP, FP, TP, DP, CRTH2 or DP2, some of them (EP1, EP3, FP, TP) existing as multiple splice variants [10,11]. PGE2 has dual uterotonic effects by action through EP1 and EP3 but may also exert an uterorelaxant effect through EP2 and EP4.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) contracts the myometrium *in vitro* and *in vivo* through activation of FP. Activation of FP [12] in the human myometrium by $PGF_{2\alpha}$ results in the elevation of intracellular calcium concentration, which, in turn, leads to contraction of the uterine smooth cell muscle [13]. FP receptor protein expression increases in the rat myometrium with advancing gestational age thus enhancing sensitivity of the myometrium to $PGF_{2\alpha}$ contractile action [14]. Likewise, FP is expressed in term human myometrium [13,15,16]. $PGF_{2\alpha}$ has been shown to induce upregulation of matrix metalloproteinase MMP1, an enzyme that breaks down collagen in cervical fibroblasts leading to cervical ripening [17]. PGF2α upregulates MMP-2 and MMP-9 and downregulates their naturally-occurring inhibitor TIMP-1 in human term decidua, thus accelerating the breakdown of collagen and the rupture of membranes [18]. PGF2α potentiates cortisol production by stimulating the enzyme 11β-hydroxysteroid dehydrogenase 1. Cortisol increases prostaglandin synthesis and decreases prostaglandin metabolism in human chorion trophoblasts thus creating a feed-forward loop in fetal membranes that may contribute to preterm birth [19]. Taken together all these findings strongly suggest that blocking FP activation might be beneficial for the control of preterm labour. Potentially, a tocolytic agent based on selective FP antagonism may be devoid of the side effects that were observed in the clinic with COX-1 and COX-2 inhibitors [20,21]. To test this hypothesis we have evaluated the effect of a recently identified orally active, potent and highly selective small molecule antagonist of the FP receptor (AS604872) in two rodent models of uterine contraction and preterm labor.

Methods

Materials

AS604872 or (2S)-3-([1,1'-biphenyl]-4-ylsulfonyl)-N-[(R)-phenyl(2-pyridinyl)methyl]-1,3-thiazolidine-2-carboxamide was chemically synthesized at the Serono Pharmaceutical Research Institute (Geneva, Switzerland). In all the in vivo experiments AS604872 was administered in NP3S (5% N-methylpyrrolidone, 25% polyethylene glycol 200, 30% polyethylene glycol 400, 20% propylene glycol, 20% saline), while ritodrine was solubilized in saline.

In vivo *experiments*

All the in vivo experiments were performed according to the European Council Directive 86/609/EEC and the Italian Health Ministry guidelines for the care and use of experimental animals (decree 116/92).

Uterine contraction model

Sprague Dawley CD (SD) BR non-pregnant (200–300 g) or late-term certified pregnant (19–21 gestation days; 350–400 g) female rats from Charles River, Calco, (Italy) were used. $PGF_{2\alpha}$- or oxytocin-induced uterine contractions in anaesthetized non-pregnant rats and spontaneous uterine contractions in anaesthetized late-term rats were all performed as described previously [22].

Mouse preterm parturition model

Time-mated, primigravid certified pregnant CD1 mice transferred from Charles River (Calco, Italy) to our animal facility at day 11 of gestation were used. Preterm labor was induced according to previous reports with some modifications [23,24]. In detail, pregnant mice at gestational day 14 or 17 were treated subcutaneously with RU486 (mifepristone) 2.5 mg/kg/5 ml at 1:00 PM. Randomly defined subgroups of animals (n = 8–10/dose) were then treated twice daily orally with AS604872 (10-30-100 mg/kg) or ritodrine (30–100 mg/kg), or the vehicle (NP3S or saline 5 ml/kg) for a total of 4 administrations (6 PM on the same day of RU486 treatment, 8 AM and 6 PM on the next

day and 8 AM on the day after). Each animal was checked for the occurrence of delivery starting from 18 hours after RU486 treatment, every three hours during the light phase, and every six hours during the dark phase. Delivery time was defined as the time of the first pup presence. The pups delivered were scored and classified as abortions, pups born alive and pups born dead. Since some of the pups may die during the time elapsing between our inspections, all the dead pups were controlled by the galenic hydrostatic pulmonary docimasy. The dead pups were dissected and a portion of the lung placed in water. Lung floating over the water indicated air content, and the ability of the pup to breathe after birth. The mean delivery time after RU486 was calculated based on the individual time of first pup delivery for each treatment.

Statistical analysis

All data are means ± SEM. Statistical analysis was performed by one-way ANOVA, followed by Tukey's test. Statistics were performed using S-Plus2000 (MathSoft Inc., Seattle, Washington, USA) and significance assumed at the 5% level.

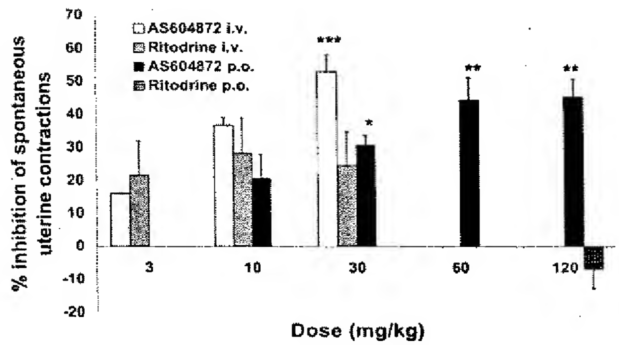

Figure 1

Effects of AS604872 and ritodrine on inhibition of spontaneous uterine contractions in pregnant rats. Anesthetized late-term pregnant rats at gestational days 19–21 were treated with AS604872 or ritodrine either by intravenous route as a 10-min infusion or orally as a single dose. The inhibition of spontaneous uterine contractions is represented in function of the dose of drugs. N = 8 animals per dose and route and drug. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ *vs* vehicle-treated group (one-way ANOVA followed by Tukey test).

Results

The potent and selective FP antagonist AS604872 (Ki human FP, 35 nM; rat FP, 158 nM, mouse, FP 323 nM; selectivity vs EP2 50-fold and >1,000-fold vs other prostanoid receptors) reduced markedly and dose-dependently the spontaneous uterine contractions in late-term pregnant rats at gestational days (GD) 19–21 (Figure 1). Intravenous injection at the two highest doses (1 and 3 mg/kg/min for 10 min infusion corresponding to a total dose of 10 and 30 mg/kg, respectively) inhibited uterine contractility by about 37 and 53%, respectively. By oral dosing, AS604872 was also able to reduce the spontaneous uterine contractions by around 28% and 32% of the pre-dose value at doses of 30 and 60 mg/kg, respectively (Figure 1). The inhibiting effect by oral route peaked at about 30 min after administration and remained at a sustained level up to the end of the observation period of 3 h (data not shown). The intravenous administration of ritodrine decreased the spontaneous uterine motility by about 25% without a dose-dependent relationship, whereas a dose of 120 mg/kg by oral route was ineffective (Figure 1).

Oral treatment with AS604872 delayed the preterm birth caused by RU486 (mifepristone) administration in pregnant mice at GD 14 or GD17. The effect was dose-dependent with a significant increase in the mean delivery time of 16 and 33 hours at doses of 30 mg/kg and 100 mg/kg, respectively, in the case of labor triggered at GD14 (Figure 2). The parturition retardation effect of AS604872 on labor induced at GD17 was less marked with a delay of 16 hours at 100 mg/kg (Figure 3). In this model AS604872 was more effective than ritodrine at GD17 whereas both compounds were comparable at GD14.

In the RU486 preterm parturition model, as an important consequence of the prolongation of gestation, some dams treated with AS604872 or ritodrine delivered viable pups (Figure 4). The proportion of live pups was higher in the GD17 than in the GD14 model. Remarkably, when labor was induced at GD17, 44% of the 100 mg/kg AS604872 treated animals delivered viable pups compared to only 11% in the group treated with 100 mg/kg ritodrine. All the other deliveries resulted in abortions.

Discussion

We have used a potent and selective small molecule antagonist of the FP receptor to decipher the role of this prostanoid receptor subtype in preterm labor and parturition. It was known that FP-deficient mice showed impaired parturition by lack of luteolysis and consequently absence of progesterone withdrawal, a necessary event to trigger labor in most animal species [25]. One key finding of our study was that blocking FP had also a clear effect on the myometrium through inhibition of uterotonic pathways in the pregnant rat model of uterine contractility. It is interesting to note that uterine activity was not fully abolished by treatment with AS604872, suggesting that other biochemical pathways are likely to be activated towards term and underlying the complex pathophysiology of preterm delivery. In the mouse parturition model, the induc-

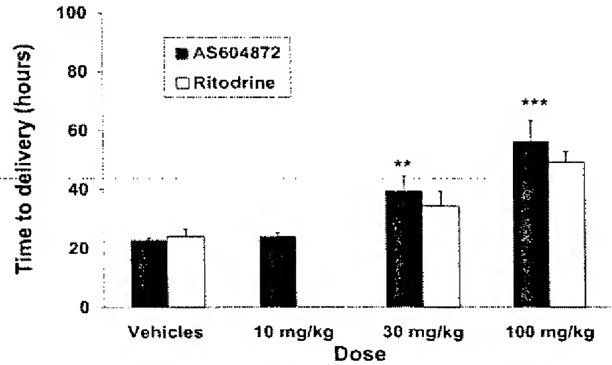

Figure 2
Delaying effect of AS604872 and ritodrine on preterm parturition in mice induced at GD14. Preterm parturition was induced by subcutaneous treatment with RU486 (mifepristone) at GD 14. Then different doses of AS604872 or ritodrine were administered orally b.i.d. and time to delivery was monitored (see Methods). Data are expressed as mean time to delivery ± SEM from the induction, n = 10.

tion of labor by the anti-progestin agent mifepristone (RU486) activates endocrine pathways and a drop in progesterone level characterized by the up-regulation of labor-associated proteins as seen in the case of idiopathic preterm labor. The FP antagonist AS604872 delayed par-

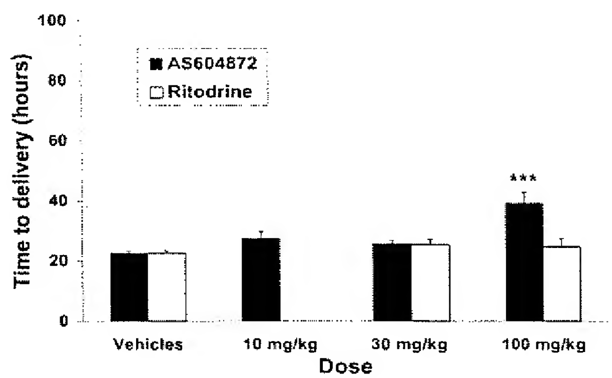

Figure 3
Delaying effect of AS604872 and ritodrine on preterm parturition in mice induced at GD17. Preterm parturition was induced by subcutaneous treatment with RU486 (mifepristone) at GD 17. Then different doses of AS604872 or ritodrine were administered orally b.i.d. and time to delivery was monitored (see Methods). Data are expressed as mean time to delivery ± SEM from the induction, n = 10.

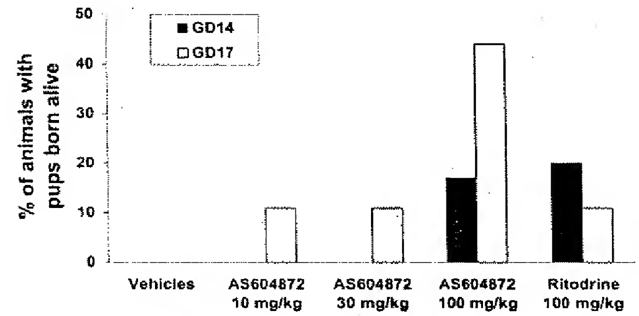

Figure 4
Effects of AS604872 and ritodrine on viability of preterm mouse pup. Data are expressed as % of dams that delivered live pups following labor induction by RU486 at GD14 or GD17 and treatment with different oral doses of AS604872 or ritodrine. The mean total number of pups (abortion, born dead and born alive) for each treatment group (9–11) was not statistically different.

turition triggered by RU486 thus indicating that FP plays a central role in preterm labor of endocrine etiology. There was a trend towards better tocolytic efficacy of AS604872 at GD14 compared to GD17. This may suggest that other biochemical pathways to labor and parturition are activated at advanced gestational age. One key observation was the enhanced viability of pups delivered by mice treated with AS604872, thus underlying the beneficial effect of prolonging gestation. In both models, AS604872 was benchmarked against the β-mimetic ritodrine that has been commonly used as tocolytic agent. There was a clear trend towards superior activity for AS604872.

Our findings support recent reports that used a non-competitive, non-selective and injectable FP peptide antagonist in sheep and human myometrial strips [26,27].

Conclusion
The tocolytic activity displayed by the selective antagonist AS604872 supports a key role for the FP receptor in the pathophysiology of premature birth and demonstrates the therapeutic potential of an FP antagonist for the treatment of preterm labor cases in which uterine hyperactivity plays a dominant role.

List of abbreviations
FP, prostaglandin F2α receptor; COX-1, COX-2, cyclooxygenase 1 or 2; PGF2α, prostaglandin F2α; GD, gestation day; MMP, matrix metallo-proteinase; TIMP, tissue inhibitor of matrix metallo-proteinase.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AC was involved in the study design, data analysis and interpretation, and drafted the manuscript. EGT performed the animal experiments and was involved in data collection, analysis and interpretation. RC was involved in the design of experiments, the data analysis and interpretation. All authors read and approved the final manuscript.

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References

1. Morrison JJ, Slattery MM: **Preterm delivery.** *Lancet* 2002, **360:**1489-1497.
2. Schwarz MK, Page P: **Preterm labor: An overview of current and emerging therapeutics.** *Curr Med Chem* 2003, **10:**1441-1468.
3. Gyetvai K, Hannah ME, Hodnett ED, Ohlsson A: **Tocolytics for preterm labor: A systematic review.** *Obstet Gynecol* 1999, **94:**869-877.
4. Berkman ND, Thorp JM Jr, Lohr KN, Carey TS, Hartmann KE, Gavin NI, *et al.*: **Tocolytic treatment for the management of preterm labor: A review of the evidence.** *Am J Obstet Gynecol* 2003, **188:**1648-1659.
5. Challis JRG, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, Whittle WL, Newnham JP: **Prostaglandins and mechanisms of preterm birth.** *Reproduction* 2002, **124:**1-17.
6. Challis JRG, Matthews SG, Gibb W, Lye SJ: **Endocrine and paracrine regulation of birth at term and preterm.** *Endocr Rev* 2000, **21:**514-550.
7. Olson DM: **The promise of prostaglandins: Have they fulfilled their potential as therapeutic targets for the delay of preterm birth?** *J Soc Gynecol Investig* 2005, **12:**466-478.
8. Slater DM, Zervou S, Thornton S: **Prostaglandins and prostanoid receptors in human pregnancy and parturition.** *J Soc Gynecol Investig* 2002, **9:**118-124.
9. Nathanielsz PW, Smith G, Wu W: **Topographical specialization of prostaglandin function in late pregnancy and at parturition in the baboon.** *Prostaglandins, Leukot Essent Fatty Acids* 2004, **70:**199-206.
10. Coleman RA, Smith WL, Narumiya S: **International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes.** *Pharmacol Rev* 1994, **46:**205-229.
11. Hata AN, Breyer RM: **Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune regulation.** *Pharmacol Ther* 2004, **103:**147-166.
12. Abramovitz M, Boie Y, Nguyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM, Grygorczyk R: **Cloning and expression of a cDNA for the human prostanoid FP receptor.** *J Biol Chem* 1994, **269:**2632-2636.
13. Senior J, Marshall K, Sangha R, Clayton JK: **In vitro characterization of prostanoid receptors on human myometrium at term pregnancy.** *Br J Pharmacol* 1993, **108:**501-506.
14. Al-Matubsi HM, Eis ALW, Brodt-Eppley J, MacPhee DJ, Ley S, Myatt L: **Expression & localization of the contractile prostaglandin F receptor in pregnant rat myometrium in late gestation, labor, postpartum.** *Biol Reprod* 2001, **65:**1029-1037.
15. Leonhardt A, Glaser A, Wegmann M, Hackenberg R, Nusing RM: **Expression of prostanoid receptors in human lower segment human myometrium.** *Prostaglandins, Leukot Essent Fatty Acids* 2003, **69:**307-313.
16. Grigsby PL, Sooranna SR, Adu-Amankwa B, Pitzer B, Brokman DE, Johnson MR, Myatt L: **Regional expression of prostaglandin E2 and F2alpha receptors in human myometrium, amnion and choriodecidua with advancing gestation and labor.** *Biol Reprod* 2006, **75:**297-305.
17. Yoshida M, Nagawa N, Itoh H, Yura S, Takemura M, Wada Y, Sato T, Itoh A, Fujii S: **Prostaglandin F2α, cytokines and cyclic mechanical stretch augment matrix metalloproteinase-1 secretion from cultured human uterine cervical fibroblast cells.** *Mol Hum Reprod* 2002, **8:**681-687.
18. Ulug U, Goldman S, Ben-Shlomo I, Shalev E: **Matrix metalloproteinase MMP-2 and MMP-9 and threir inhibitor TIMP-1, in human term decidua and fetal membranes: the effect of prostaglandin F2α and indomethacin.** *Mol Hum Reprod* 2001, **7:**1187-1193.
19. Alfaidy N, Xiong ZG, Myatt L, Lye SJ, Macdonald JF, Challis JRG: **Prostaglandin F2α potentiates cortisol production by stimulating 11β-hydroxysteroid dehydrogenase 1: A novel feedback loop that may contribute to human labor.** *J Clin Endocrinol Metab* 2001, **86:**5585-5592.
20. Norton ME, Merrill J, Cooper B, Kuller JA, Clyman RI: **Neonatal complications after the administration of indomethacin for preterm labor.** *New Engl J Med* 1993, **329:**1602-1607.
21. Peruzzi L, Gianoglio B, Porcellini MG, Coppo R: **Neonatal end-stage renal failure associated with maternal ingestion of COX-1 selective inhibitor nimesulide as tocolytic.** *New Engl J Med* 1999, **354:**1615.
22. Cirillo R, Gillio Tos E, Schwarz MK, Quattropani A, Scheer A, Missotten M, Dorbais J, Nichols A, Borrelli F, Giachetti C, Golzio L, Marinelli P, Thomas RJ, Chevillard C, Laurent F, Portet K, Barberis C, Chollet A: **Pharmacology of (2S, 4Z)-N-[(2S)-2-hydroxy-2-phenylethyl]-4-(methoxyimino)-1-[(2'-methyl[1,1'-biphenyl]-4-yl)carbonyl]-2-pyrrolidinecarboxamide, a new potent and selective non-peptide antagonist of the oxytocin receptor.** *J Pharmacol Exp Ther* 2003, **306:**253-261.
23. Dudley DJ, Branch DW, Edwin SS, Mitchell MD: **Induction of preterm birth in mice by RU486.** *Biol Reprod* 1996, **55:**992-995.
24. Garfield RE, Gasc JM, Baulieu EE: **Effects of the antiprogesterone RU 486 on preterm birth in rat.** *Am J Obstet Gynecol* 1987, **157:**1281-1285.
25. Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T, Oida H, Yoshida N, Tanaka T, Katsuyama M, Hasumoto K, Murata T, Hirata M, Ushikubi F, Negishi M, Ichikawa A, Narumiya S: **Failure of parturition in mice lacking the prostaglandin F receptor.** *Science* 1997, **277:**681-683.
26. Hirst JJ, Parkington HC, Young IR, Palliser HK, Peri KG, Olson DM: **Delay of preterm birth in sheep by THG113.31, a prostaglandin F2α receptor antagonist.** *Am J Obstet Gynecol* 2005, **193:**256-266.
27. Friel AM, O'Reilly MW, Sexton DJ, Morrison JJ: **Specific PGF2a receptor (FP) antagonism and human uterine contractility in vitro.** *BJOG Int J Obstet Gynaecol* 2005, **112:**1034-1042.

Annex 3: Sales et al., "A Novel Angiogenic Role for Prostaglandin $F_{2\alpha}$-FP Receptor Interaction in Human Endometrial Adenocarcinomas," Cancer Res. 65:17 (2005).

A Novel Angiogenic Role for Prostaglandin $F_{2\alpha}$-FP Receptor Interaction in Human Endometrial Adenocarcinomas

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Abstract

Prostaglandins have been implicated in several neovascular diseases. In the present study, we found elevated FP receptor and vascular endothelial growth factor (VEGF) expression colocalized in glandular epithelial and vascular cells lining the blood vessels in endometrial adenocarcinomas. We investigated the signaling pathways activated by the FP receptor and their role in modulating VEGF expression in endometrial adenocarcinoma (Ishikawa) cells. Ishikawa cells were stably transfected with FP receptor cDNA in the sense or antisense orientations. Treatment of Ishikawa cells with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) rapidly induced transphosphorylation of the epidermal growth factor receptor (EGFR) and phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 via the FP receptor. Activation of EGFR-Ras-mitogen-activated protein kinase/ERK kinase (MEK) signaling via the FP receptor resulted in an increase in VEGF promoter activity, expression of VEGF mRNA, and secretion of VEGF protein. These effects of $PGF_{2\alpha}$ on the FP receptor could be abolished by treatment of cells with a specific FP receptor antagonist, chemical inhibitors of c-Src, matrix metalloproteinase, and EGFR kinase or by inactivation of signaling with dominant-negative mutant isoforms of EGFR, Ras, or MEK or with small inhibitory RNA oligonucleotides targeted against the EGFR. Finally, we confirmed that $PGF_{2\alpha}$ could potentiate angiogenesis in endometrial adenocarcinoma explants by transactivation of the EGFR and induction of VEGF mRNA expression. (Cancer Res 2005; 65(17): 7707-16)

Introduction

Over the past decade, a role has been established for cyclooxygenase (COX) enzymes and prostaglandins in the development and progression of inflammation and cancer (1, 2). Of particular significance to female reproductive health are the observations that reproductive tract cancers, such as endometrial adenocarcinoma and cervical carcinoma, which highly express COX enzymes, also contain elevated levels of prostaglandins and expression and signaling of prostanoid G protein–coupled receptors (GPCR; refs. 3–6). These findings suggest that aberrant prostanoid receptor signaling may be key toward the regulation or progression of female reproductive tract pathologies, such as endometrial adenocarcinoma.

In the reproductive tract, the E and F series of prostaglandins are the most abundantly biosynthesized prostanoids, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a major metabolite of COX enzymes in human endometrium (7, 8). Currently, there are three known isoforms of COX enzyme, COX-1, COX-2, and COX-3 (a splice variant of COX-1; refs. 3, 9). $PGF_{2\alpha}$ is biosynthesized from arachidonic acid by a series of oxidation steps by COX enzymes and PGF synthase, respectively (10). Following biosynthesis, $PGF_{2\alpha}$ is transported out of the cell by a prostaglandin transporter (11) where it exerts an autocrine/paracrine function through GPCR-mediated interaction. The GPCR for the human $PGF_{2\alpha}$ (FP receptor) has been cloned, and its activation leads to coupling to the G protein G_q and release of inositol-1,4,5-trisphosphate and diacylglycerol (12).

Recently, we have reported elevated expression of the FP receptor in human endometrial adenocarcinomas and ascertained a role for $PGF_{2\alpha}$ in enhancing the proliferation of endometrial adenocarcinoma cells (6), confirming an autocrine/paracrine regulation of neoplastic cell function by $PGF_{2\alpha}$-FP receptor interaction. Prostanoid biosynthesis and signaling, including $PGF_{2\alpha}$, have been implicated in numerous endometrial pathologies, including excessive menstrual bleeding (menorrhagia), endometriosis, and dysmenorrhea (7, 13, 14).

Like many other solid tumors, the growth and proliferation of endometrial adenocarcinoma is dependent on angiogenesis, the formation of new blood vessels from the preexisting vascular bed (15). Of the numerous proangiogenic factors reported to date, vascular endothelial growth factor (VEGF) is the progenitor in the mediation of tumor angiogenesis (16). Elevated expression of VEGF in human tumor biopsies has been reported in various carcinomas, including endometrial adenocarcinoma (17). Recently, a role for the E-series prostanoid receptors in angiogenesis has been established (for review, see ref. 3). However, little is known about FP receptor signaling to angiogenic factors, such as VEGF.

In this study, we investigated the potential role of elevated FP receptor expression and signaling in modulating vascular function in endometrial adenocarcinomas. We found elevated FP receptor and VEGF expression in endometrial adenocarcinomas, colocalized in glandular epithelial and endothelial cells. We investigated the signal transduction pathways associated with VEGF expression via the FP receptor using an endometrial adenocarcinoma (Ishikawa) cell model system. We found that $PGF_{2\alpha}$ rapidly augments the activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway in a c-Src-, matrix metalloproteinase (MMP)–, epidermal growth factor receptor (EGFR)–, and Ras-dependent manner via the FP receptor. Activation of the EGFR-Ras-mitogen-activated protein kinase (MAPK)/ERK kinase (MEK)-ERK1/2 pathway resulted in an increase in VEGF promoter activity, VEGF mRNA expression, and

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secretion of VEGF protein. Finally, using human endometrial adenocarcinoma explants, we confirmed that $PGF_{2\alpha}$ could potentiate tumorigenesis *in situ* by transactivating the EGFR and inducing the expression of VEGF to promote angiogenesis.

Materials and Methods

Reagents

Culture medium was purchased from Life Technologies (Paisley, United Kingdom). Penicillin-streptomycin and FCS were purchased from PAA Laboratories (Middlesex, United Kingdom). The c-Myc (sc-40), EGFR (sc-03), phospho-EGFR (sc-12351), and ERK (sc-93) antibodies were purchased from Santa Cruz Biotechnology/Autogen-Bioclear (Wiltshire, United Kingdom). The FP receptor antibody (101802) was purchased from Cayman Chemical Co./Alexis Corp. (Nottingham, United Kingdom). Anti-phospho-p42/p44 ERK (9101), phospho-p38 (9211), total p38 (9212), phospho-c-Jun NH_2-terminal kinase (JNK; 9251), and total JNK (9252) antibodies were purchased from Cell Signaling Technologies/New England Biolabs (Herts, United Kingdom). The CD31 antibody was purchased from DAKO Corp. (High Wycombe, United Kingdom). Alkaline phosphatase secondary antibodies, indomethacin, PBS, bovine serum albumin, and $PGF_{2\alpha}$ were purchased from Sigma Chemical Co. (Dorset, United Kingdom). ECF chemiluminescence system was purchased from Amersham Biosciences (Buckinghamshire, United Kingdom). PD98059 (18.7 mmol/L stock in DMSO), PP2 (10 mmol/L stock in DMSO), GM6001 (10 mmol/L stock in DMSO), CF109203X (10 mmol/L stock in DMSO), and AG1478 (10 mmol/L stock in DMSO) were purchased from Calbiochem (Nottingham, United Kingdom) and stored at −20°C.

Patients and Tissue Collection

Endometrial adenocarcinoma tissue (n = 25) was collected from women undergoing hysterectomy who had been prediagnosed to have adenocarcinoma of the uterus. All women were postmenopausal and had received no treatment before surgery. The ages of the patients ranged from 50 to 71 years with a median age of 60.5 years. Hysterectomy specimens for adenocarcinoma were collected from the operating theatre and placed on ice. With minimal delay, the specimens were opened by a gynecologic pathologist. Small samples (~5 mm-3 cm) of polypoidal adenocarcinoma tissue were collected from the endometrial lumen, transferred into neutral-buffered formalin (and wax embedded for immunofluorescence studies), snap frozen in dry ice, and stored at −70°C (for RNA extraction), and placed in RPMI 1640 containing 2 mmol/L L-glutamine, 100 units penicillin, 100 μg/mL streptomycin, and 3 μg/mL indomethacin (to inhibit endogenous COX activity) for *in vitro* culture. The diagnosis of adenocarcinoma was confirmed histologically in all cases and the percentage of tumor cells to stroma was estimated to be ~75%:25%. Normal endometrial tissue (n = 10) at different stages of the menstrual cycle was collected from women undergoing surgery for minor gynecologic procedures with no underlying endometrial pathology with an endometrial suction curette (Pipelle, Laboratoire CCD, Paris, France) from women with regular menstrual cycles (25-35 days) and processed exactly as described above. The ages of the control women ranged from 21 to 39 years with a median age of 30.5 years. None of the control women had received a hormonal preparation in the 3 months preceding biopsy collection. Biopsies were dated according to the stated last menstrual period and confirmed by histologic assessment according to the criteria of Noyes et al. (18). Ethical approval was obtained from Lothian Research Ethics Committee and written informed consent was obtained from all subjects before tissue collection.

Cell Culture

Ishikawa endometrial adenocarcinoma cells (European Collection of Cell Culture, Wiltshire, United Kingdom) were maintained as described previously (6). Stable FP transfectant cells were maintained under the same conditions with the addition of a maintenance dose of 200 μg/mL G418.

FP Receptor Amplification and Cell Transfections

The FP receptor cDNA clone and stable cell lines were constructed by Cytomix Ltd. (Cambridge, United Kingdom). The FP receptor cDNA was amplified from a human lung cDNA library from the Genbank accession no. NM_000959 by PCR using the proofreading polymerase *Pfu* (Stratagene, Amsterdam, The Netherlands). The product was directionally cloned into the mammalian expression vector gWIZ3.0 (Gene Therapy Systems, Cambridge, United Kingdom), in both sense and antisense directions, and the orientation of the construct was confirmed by automated DNA sequencing. The FP receptor cDNA was transfected into Ishikawa cells at passage 10 in the sense and antisense directions. Control cells transfected with pcDNA6/V5/His/LacZ (Invitrogen, De Schelp, The Netherlands) cDNA and assayed with β-galactosidase were used to determine a transfection efficiency of 45 ± 5%. Individual cell populations were selected for with addition of 800 μg/mL G418. Full selection was confirmed by the 100% death of nontransfected control cells, and 11 single FP receptor sense and antisense clones were selected and expanded for Western blot analysis. The clones with the highest level of FP receptor expression [FP sense (FPS)] and lowest receptor expression [FP antisense (FPAS)] were expanded and stored in liquid nitrogen. Based on the relative quantification of FP receptor expression, two sense clones (S32 and S22) and two antisense clones (A14 and A19) exhibiting similar phenotypic and biochemical alterations were supplied. The results of our studies using the S32 and A19 clones are presented here.

Immunofluorescent Microscopy

Cells. Approximately 10,000 wild-type (WT), FPS, and FPAS cells were seeded in chamber slides, fixed in methanol, washed in PBS, and blocked using 5% normal swine serum. Subsequently, the cells were incubated with polyclonal rabbit anti–FP receptor antibody at a dilution of 1:50 at 4°C for 18 hours followed by swine anti-rabbit tetramethylrhodamine isothiocyanate (DAKO) at 25°C for 20 minutes and counterstained with To-Pro-3 (Molecular Probes, Inc., Eugene, OR) at a dilution of 1:2,000 for 2 minutes. Cells were then mounted in Permafluor (Immunotech-Coulter, Buckinghamshire, United Kingdom) and coverslipped. Control cells were incubated with rabbit IgG.

Tissues. FP receptor and VEGF or CD31 protein expression were colocalized in endometrial adenocarcinomas (n = 12) by dual immunofluorescence immunohistochemistry. Tissue sections were prepared as described previously (6) and blocked using 5% normal horse (for FP/VEGF) or normal goat (for FP/CD31) serum diluted in PBS. Subsequently, sections were incubated with either goat anti-VEGF antibody at a dilution of 1:50 or mouse anti-CD31 antibody (1:30) for 18 hours at 4°C. Control sections were incubated with goat or mouse IgG, respectively. Thereafter, sections were washed with PBS and incubated with biotinylated horse anti-goat for FP/VEGF or goat anti-mouse IgG (DAKO) followed by incubation with the fluorochrome streptavidin Alexa Fluor 488 (Molecular Probes) diluted 1:200 in PBS. Sections were reblocked with 5% normal goat serum diluted in PBS and incubated with rabbit anti–FP receptor antibody at a dilution of 1:100 at 4°C for 18 hours. Control sections were incubated with rabbit IgG. Thereafter, the cells were washed in PBS and incubated with the fluorochrome streptavidin Alexa Fluor 546 diluted 1:200 in PBS at 25°C for 20 minutes. Cells were mounted in Permafluor and coverslipped. Fluorescent images were visualized and photographed using a Carl Zeiss (Jena, Germany) laser scanning microscope LM510.

Protein Extraction

Cells. For EGFR transactivation studies, 3×10^6 cells were seeded in 10 cm dishes, and for MAPK studies, 1×10^6 cells were seeded in 5 cm dishes. The following day cells were incubated in serum-free culture medium and 8.4 μmol/L indomethacin (a dual COX enzyme inhibitor used to inhibit endogenous prostanoid biosynthesis) for at least 16 hours. The next day, cells were pretreated with specific inhibitors for c-Src (PP2, 10 μmol/L), MMP (GM6001, 10 μmol/L), EGFR kinase (AG1478, 100 nmol/L), or MEK (PD98059, 50 μmol/L) or FP receptor antagonist AL8810 (Calbiochem) or vehicle for 1 hour before stimulation with 100 nmol/L $PGF_{2\alpha}$ (for the period specified in the figure legends) or vehicle. Following stimulation with $PGF_{2\alpha}$, proteins were extracted and quantified as described previously (6). The protein content in the supernatant fraction was determined using protein assay kits (Bio-Rad, Hemel Hempstead, United Kingdom).

Tissue. For EGFR transactivation studies, carcinoma tissue (n = 4) explants were finely chopped using a sterile scalpel blade and incubated in

serum-free medium for at least 12 hours containing penicillin/streptomycin (as described previously) and 8.4 μmol/L indomethacin. The next day, tissue was pretreated with 50 μmol/L AL8810 or 100 nmol/L AG1478 for 1 hour before stimulation with 100 nmol/L $PGF_{2\alpha}$ for 10 minutes. Following stimulation with $PGF_{2\alpha}$, tissue was washed with PBS and protein was harvested by homogenization in protein lysis buffer, clarified by centrifugation, and assayed as described above before immunoprecipitation and/or Western blot analysis.

Immunoprecipitation and Western Blot Analysis

For immunoprecipitation studies, equal amounts of protein were incubated with specific EGFR or c-Myc antibody preconjugated to protein A-Sepharose overnight at 4°C with gentle rotation. Beads were washed extensively with lysis buffer and immune complexes were solubilized in Laemmli buffer [125 mmol/L Tris-HCl (pH 6.8), 4% SDS, 5% 2-mercaptoethanol, 20% glycerol, 0.05% bromophenol blue] and then boiled for 5 minutes. For FP receptor expression in cells and MAPK studies, a total of 50 μg of protein was resuspended in 20 μL Laemmli buffer. Proteins were resolved and immunoblotted as described previously (6) and incubated with specific primary and alkaline phosphatase–conjugated secondary antibodies. Immunoreactive proteins were visualized by the ECF chemiluminescence system according to the manufacturer's instructions. Proteins were revealed and quantified by PhosphorImager analysis using the Typhoon 9400 system (Molecular Dynamics/Amersham Biosciences). Relative density in immunoblots was calculated by dividing the value obtained from the phosphorylated blots by the value obtained from total protein blots and expressed as fold above vehicle controls. All data are presented as mean ± SE.

Total Inositol Phosphate Assays

Total inositol phosphate production was measured in Ishikawa WT, FPS, and FPAS cells treated either with vehicle, 100 nmol/L $PGF_{2\alpha}$, or $PGF_{2\alpha}$ and increasing doses of AL8810 and assayed as described previously (19). Data are presented as mean ± SE.

Taqman Quantitative Reverse Transcription-PCR

FP receptor and VEGF (n = 25 carcinoma and n = 10 normal tissues) expression in endometrial tissues and VEGF expression in FPS cells was measured by quantitative reverse transcription-PCR (RT-PCR) analysis as described previously (20). Cells were synchronized by serum withdrawal for at least 12 hours in serum-free medium containing 8.4 μmol/L indomethacin. Carcinoma explants were finely chopped using a sterile scalpel blade and incubated in serum-free medium for at least 12 hours. Thereafter, medium was removed and replaced with fresh medium containing indomethacin with either 100 nmol/L $PGF_{2\alpha}$, vehicle, or 100 nmol/L $PGF_{2\alpha}$ and chemical inhibitor for the time indicated in the figure legends. RNA was extracted using Tri-reagent (Sigma Chemical) following the manufacturer's guidelines. Once extracted and quantified, RNA samples were reverse transcribed and subjected to RT-PCR analysis using an ABI Prism 7700 as described previously (6, 20). VEGF and FP primers and probe for quantitative PCR were designed using the PRIMER express program (PE Applied Biosystems, Warrington, United Kingdom) as described previously (6, 20). Data were analyzed and processed using Sequence Detector version 1.6.3 (PE Applied Biosystems). Expression of VEGF/FP was normalized to RNA loading for each sample using the 18S rRNA as an internal standard. Results are expressed as fold increase above vehicle treated cells.

Transient Transfections

Cell signaling to ERK1/2 was determined using dominant-negative (DN) isoforms targeted against Ras GTPase, EGFR, and MEK. The transfection efficiency of Ishikawa cells had been determined as described earlier and the plasmids for the various ERK cascade members were described recently (21–24). In addition, small interfering RNAs (siRNA) were used to abolish EGFR expression and function using a siRNA/siAB assay kit (Upstate, Milton Keynes, United Kingdom). Transfection efficiency of siRNA experiments was determined by immunofluorescence microscopy as 48 ± 5% using a fluorescein-labeled siRNA. Briefly, Ishikawa sense cells were seeded to a density of 5×10^5 cells per well in 6 cm dishes and exposed to either 300 nmol/L EGFR siRNA or 100 nmol/L control random siRNA or 5 μg c-Myc-ERK1/2 and DN-Ras, DN-EGFR, DN-MEK, or empty vector plasmid (pcDNA3) in the presence of Superfect (Qiagen, Crawley, United Kingdom) for 4 hours and then cultured for 24 hours in complete medium. Thereafter, cells were serum starved for 24 hours in medium containing indomethacin and then exposed to 100 nmol/L $PGF_{2\alpha}$ or vehicle for 10 minutes. Thereafter, cells were lysed and protein was quantified and subjected to Western blot analysis as described above.

VEGF ELISA

Secreted VEGF was measured by ELISA. Cells were first synchronized by serum withdrawal for 12 hours in serum-free medium containing 8.4 μmol/L indomethacin. Thereafter, medium was removed and replaced with fresh serum-free medium containing indomethacin and vehicle, 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and AL8810 for 24 hours. Culture medium was removed and VEGF protein was measured using a Human VEGF ELISA kit according to the manufacturer's instruction (Oncogene, Nottingham, United Kingdom). Data are expressed as fold increase where the amount of VEGF secreted in treated cells is divided by the amount secreted in cells treated with the vehicle. The data are presented as mean ± SE from three independent experiments.

VEGF Luciferase Reporter Assays

The VEGF reporter plasmid (kindly supplied by Prof. Keping Xie, Department of Gastrointestinal Medical Oncology and Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX; ref. 25) containing the firefly luciferase reporter was cotransfected into Ishikawa cells in triplicate with an internal control pRL-TK (containing the *Renilla* luciferase coding sequence; Promega, Southampton, United Kingdom) and either control vector (pcDNA3.0) or vector encoding a DN-Ras, DN-EGFR, and DN-MEK. Cells were transfected using Superfect for 6 hours. The following day, the cells were serum starved for at least 18 hours with indomethacin before stimulation for 8 hours with vehicle, $PGF_{2\alpha}$, or $PGF_{2\alpha}$ and AL8810. The activity of both firefly and *Renilla* luciferase was determined using the dual luciferase assay kit. Fold increase in luciferase activity was calculated by dividing the relative luciferase activity in cells treated with $PGF_{2\alpha}$ by the relative luciferase activity in cells treated with vehicle.

Statistics

Where appropriate, data were subjected to statistical analysis with ANOVA and Fisher's protected least significant difference tests (Statview 5.0, Abacus Concepts, Inc., Carpenteria, CA).

Results

FP receptor and vascular endothelial growth factor expression in endometrial adenocarcinoma and normal endometrium. The expression of FP receptor and VEGF mRNA was significantly up-regulated in all cases of endometrial adenocarcinoma investigated compared with normal endometrium (P < 0.05) as determined by Taqman quantitative RT-PCR analysis (Fig. 1*A*). No correlation was observed between levels of expression of FP receptor and VEGF mRNA and grade or stage of carcinoma. The relative expression of FP receptor and VEGF in endometrial adenocarcinomas was determined to be 53.7 ± 16.9 and 32.5 ± 10.2, respectively, compared with expression in normal endometrium (0.4 ± 0.12 and 1.12 ± 0.25 for FP receptor and VEGF, respectively).

Colocalization of FP receptor with vascular endothelial growth factor or CD31 in endometrial adenocarcinomas. FP receptor and VEGF expression was colocalized in endometrial adenocarcinomas by dual immunofluorescence immunohistochemistry (Fig. 1*B*). FP receptor expression and VEGF expression were localized together (FP/VEGF) in the glandular epithelial and endothelial compartment in all poorly, moderately, and well-differentiated endometrial adenocarcinomas. To confirm that FP receptor was localized to the endothelial cells of blood vessels, we did dual immunofluorescence immunohistochemistry on tissue

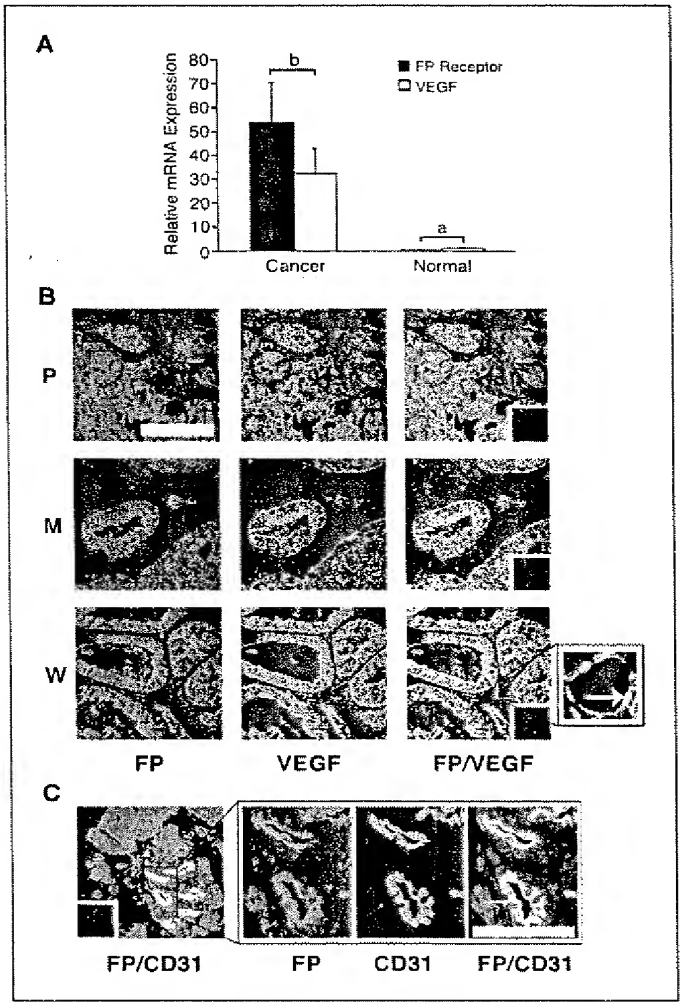

Figure 1. *A,* relative mRNA expression of FP receptor and VEGF in endometrial adenocarcinoma (n = 25) and normal endometrium (n = 10) as determined by real-time quantitative RT-PCR analysis. *B,* localization of the site of expression of FP receptor (*red*) and VEGF (*green*) and colocalization of FP receptor with VEGF (merged; *yellow*) in poorly (*P*), moderately (*M*), and well-differentiated (*W*) endometrial adenocarcinomas, respectively. *Arrow,* blood vessel under high magnification. *Bar,* 100 μm. *C,* localization of the site of expression of FP receptor (*red*) and CD31 (*green*) and colocalization of FP receptor with CD31 (merged; *yellow*) in the endothelial cells of the blood vessels. Representative sample of a moderately differentiated adenocarcinoma. *Insets,* negative controls. *Bar,* 10 μm. $P < 0.05$, *b* is significantly different from *a*.

sections using antibodies raised against the FP receptor and CD31 endothelial cell marker (Fig. 1*C*). FP receptor (FP) colocalized (FP/CD31) in the blood vessels with CD31 in all endometrial adenocarcinomas. Incubating sections with nonimmune IgG from the host species abolished the immunoreactivity.

FP receptor expression in Ishikawa cells. FP receptor expression was assessed in stably transfected Ishikawa cells overexpressing the FP receptor in the sense (FPS) and antisense (FPAS) orientations. Western blot analysis (Fig. 2*A*) and immunofluorescence microscopy (Fig. 2*B*) showed elevated FP receptor expression in FPS cells and reduced FP expression in FPAS cells compared with WT cells. FP receptor expression was localized to the plasma membrane (Fig. 2*B*) and was abolished by incubating cells with rabbit IgG in place of antibody (Fig. 2*B*, control representative of FPS cells, and Fig. 2*C*). Quantitative RT-PCR analysis was then used to compare FP receptor expression in WT and FPS Ishikawa cells with FP receptor expression detected in normal endometrium and endometrial adenocarcinomas *in vivo*. As shown in Fig. 2*C*, the relative FP receptor mRNA expression in FPS cells and adenocarcinoma tissues was significantly greater than the FP receptor expression in WT Ishikawa cells or normal endometrial tissue ($P < 0.05$). FP receptor couples to G_q resulting in increased formation of inositol phosphate. We measured total inositol phosphate accumulation in WT, FPS, and FPAS cells in response to administration of 100 nmol/L $PGF_{2\alpha}$. A significant increase in inositol phosphate accumulation was observed in FPS cells compared with WT and FPAS cells following treatment with $PGF_{2\alpha}$ ($P < 0.05$; Fig. 2*D*). Treatment of FPS cells with the selective

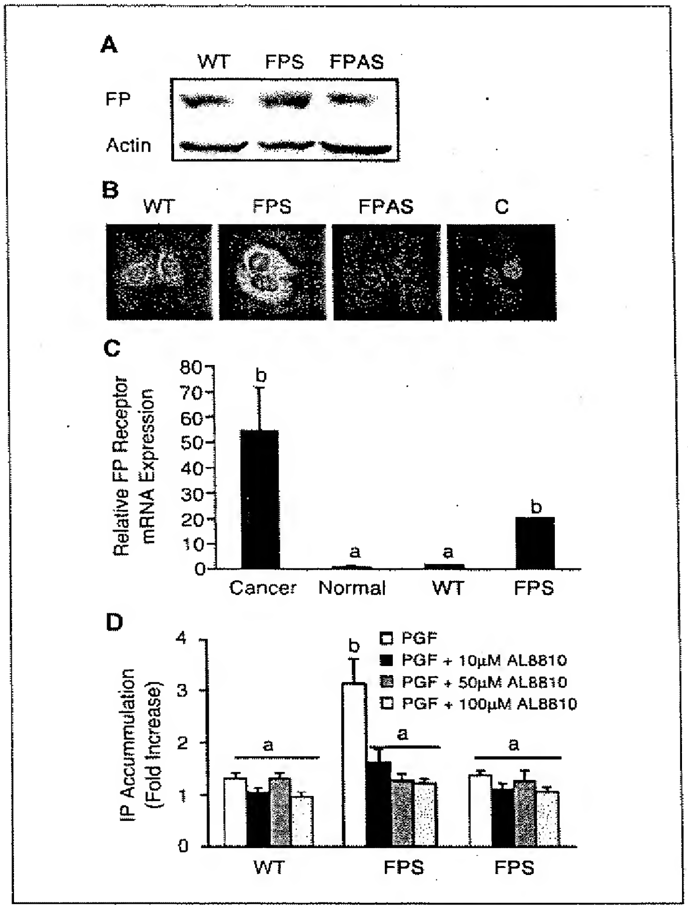

Figure 2. *A,* representative Western blot of FP receptor expression in WT, FPS, and FPAS cells. Cells were immunoblotted with specific FP receptor antibody and normalized for loading against β-actin on the same blot. *B,* immunofluorescence microscopy done on WT, FPS, and FPAS cells using a specific FP receptor primary antibody. Control cells (*C*) were incubated with normal IgG in place of primary antibody (representative FPS cells incubated with IgG). *C,* relative expression of FP receptor mRNA in endometrial adenocarcinoma (n = 25) and normal endometrial tissues (n = 10) compared with FP receptor mRNA expression in Ishikawa WT and FPS cell lines. *D,* inositol phosphate (*IP*) accumulation was assessed in WT, FPS, and FPAS cells in response to administration of vehicle, 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and increasing doses of specific FP receptor antagonist AL8810. *Columns,* mean of three independent experiments; *bars,* SE. $P < 0.05$, *b* is significantly different from *a*.

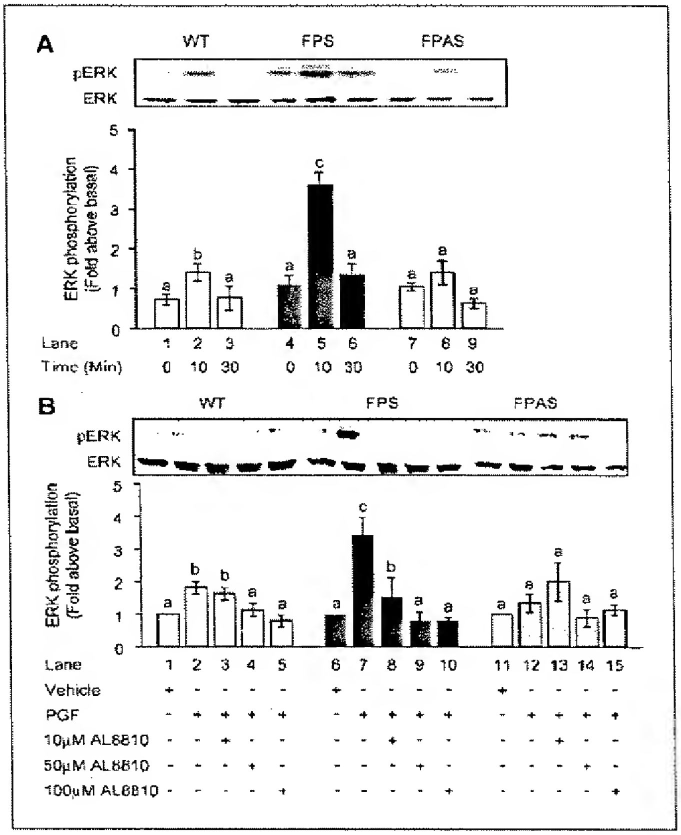

Figure 3. Effect of $PGF_{2\alpha}$ on ERK1/2 signaling in WT, FPS, and FPAS Ishikawa cells. *A*, Ishikawa cells were stimulated with 100 nmol/L $PGF_{2\alpha}$ for 0, 10, and 30 minutes and subjected to immunoblot analysis using antibody against phosphorylated ERK1/2 (*top*). The total amount of ERK in cell lysates was determined by reprobing the same blot with antibody recognizing total protein (*bottom*). *B*, Ishikawa WT, FPS, and FPAS cells were pretreated for 1 hour with increasing doses of specific FP receptor antagonist (AL8810) or vehicle followed by stimulation with vehicle, 100 nmol/L $PGF_{2\alpha}$, or $PGF_{2\alpha}$ and antagonist for 10 minutes. For each, a representative Western blot is shown, with semiquantitative analysis of ERK phosphorylation determined as described in Materials and Methods. *Columns*, mean of four independent experiments; *bars*, SE. $P < 0.05$, *b* is significantly different from *a*; $P < 0.01$, *c* is significantly different from *a* and *b*. –, absence of agent; +, presence of agent.

FP receptor antagonist AL8810 (26) dose-dependently reduced the agonist-induced inositol phosphate accumulation ($P < 0.05$; Fig. 2*D*).

Prostaglandin $F_{2\alpha}$-FP receptor activation of mitogen-activated protein kinase signaling. The effect of $PGF_{2\alpha}$ on the activation of the downstream MAPK signaling pathways (ERK1/2, p38, and JNK) was determined after treatment of WT, FPS, and FPAS Ishikawa cells with 100 nmol/L $PGF_{2\alpha}$. Stimulation of Ishikawa cells with $PGF_{2\alpha}$ caused a rapid and augmented activation of ERK1/2 in FPS compared with WT and FPAS cells (Fig. 3*A*). The peak of ERK1/2 activation was observed after 10 minutes in Ishikawa FPS treated with 100 nmol/L $PGF_{2\alpha}$ (Fig. 3*A*, *lane 5*) compared with cells treated with vehicle alone (Fig. 3*A*, *lane 4*). No significant phosphorylation of p38 or JNK MAPK was observed in WT, FPS, or FPAS cells following treatment of cells with 100 nmol/L $PGF_{2\alpha}$ within the period of activation of ERK1/2 (data not shown). Cotreatment of Ishikawa WT, FPS, and FPAS cells with the selective FP receptor antagonist AL8810 dose-dependently inhibited the activation of ERK1/2, indicating that the ERK1/2 activation was mediated via direct $PGF_{2\alpha}$-FP receptor interaction (Fig. 3*B*).

Prostaglandin $F_{2\alpha}$-FP receptor activation of extracellular signal-regulated kinase 1/2 signaling requires epidermal growth factor receptor kinase activity. We next evaluated the effect of the selective inhibitors of MEK (PD98059), MMP (GM6001), c-Src (PP2), and EGFR kinase (AG1478) on the $PGF_{2\alpha}$-induced activation of ERK1/2 signaling in FPS cells. Treatment of FPS cells with 100 nmol/L $PGF_{2\alpha}$ induced a rapid phosphorylation of ERK1/2 (Fig. 4*A*, *lane 2*) compared with FPS cells treated for the same period with vehicle alone (Fig. 4*A*, *lane 1*). This elevation in ERK1/2 phosphorylation was abolished by treatment of FPS cells with PD98059 (Fig. 4*A*, *lane 3*), GM6001 (Fig. 4*A*, *lane 4*), PP2 (Fig. 4*A*, *lane 5*), or AG1478 (Fig. 4*A*, *lane 6*). These results implicate the involvement of c-Src, MMP, and EGFR in $PGF_{2\alpha}$-FP receptor signaling to ERK1/2. To further explore a role for the EGFR and the small GTPase Ras in the activation of ERK1/2 by $PGF_{2\alpha}$, we cotransfected FPS cells with the cDNA for c-Myc-tagged ERK with either DN-Ras, DN-EGFR, and DN-MEK or an empty vector (pcDNA3.0). FPS cells were then treated with either vehicle, 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and AL8810 for 10 minutes. The tagged ERK was immunoprecipitated with anti-c-Myc antibodies and ERK activity was determined by Western blotting as described in Materials and Methods. $PGF_{2\alpha}$ rapidly phosphorylated ERK1/2 in FPS cells (Fig. 4*B*, *lane 2*). This activation of ERK1/2 was abolished by cotreatment with the FP receptor antagonist (AL8810, Fig. 4*B*, *lane 3*) or by cotransfection with the DN-Ras (Fig. 4*B*, *lane 4*), DN-EGFR (Fig. 4*B*, *lane 5*), and DN-MEK (Fig. 4*B*, *lane 6*). Preliminary proteomic phosphosite array studies (Kinexus Bioinformatics Corp., Vancouver, British Columbia, Canada) done by our laboratory showed that the predominant isoforms of protein kinase C (PKC) activated by prostanoids in our Ishikawa cells were PKCα, PKCβ1, and PKCβ2.[4] We investigated whether $PGF_{2\alpha}$-FP signaling to ERK1/2 occurred via activation of these PKC isoforms by cotransfecting FPS cells with the cDNA for c-Myc-tagged ERK with either DN-PKCα, DN-PKCβ1, or DN-PKCβ2 or an empty vector (pcDNA3.0). FPS cells were then treated with either vehicle or 100 nmol/L $PGF_{2\alpha}$ for 10 minutes and the cells were immunoprecipitated and immunoblotted as described above. No reduction in ERK phosphorylation was observed in cells transfected with the DN-PKCα (Fig. 4*C*, *lane 3*), DN-PKCβ1 (Fig. 4*C*, *lane 4*), or DN-PKCβ2 (Fig. 4*C*, *lane 5*), implicating that $PGF_{2\alpha}$-FP receptor signaling to ERK1/2 did not occur via these PKC isoforms (Fig. 4*C*). We further coincubated FPS cells with the specific PKC inhibitor GF109203X (data not shown), which also did not abolish the $PGF_{2\alpha}$-induced activation of ERK1/2, confirming that PKC was not involved in the $PGF_{2\alpha}$-induced activation of ERK1/2.

The involvement of the EGFR in transducing ERK1/2 signaling in FPS cells was confirmed using RNA interference. FPS cells were transfected with either control random siRNA or siRNA duplexes targeted against the EGFR. FPS cells were treated with either vehicle or 100 nmol/L $PGF_{2\alpha}$ for 10 minutes. ERK1/2 phosphorylation was significantly reduced in cells transfected with EGFR siRNA (Fig. 4*D*, *lane 3*) compared with FPS cells transfected with control random siRNA (Fig. 4*D*, *lane 2*). EGFR levels in cells

[4] K.J. Sales and H.N. Jabbour, unpublished observation.

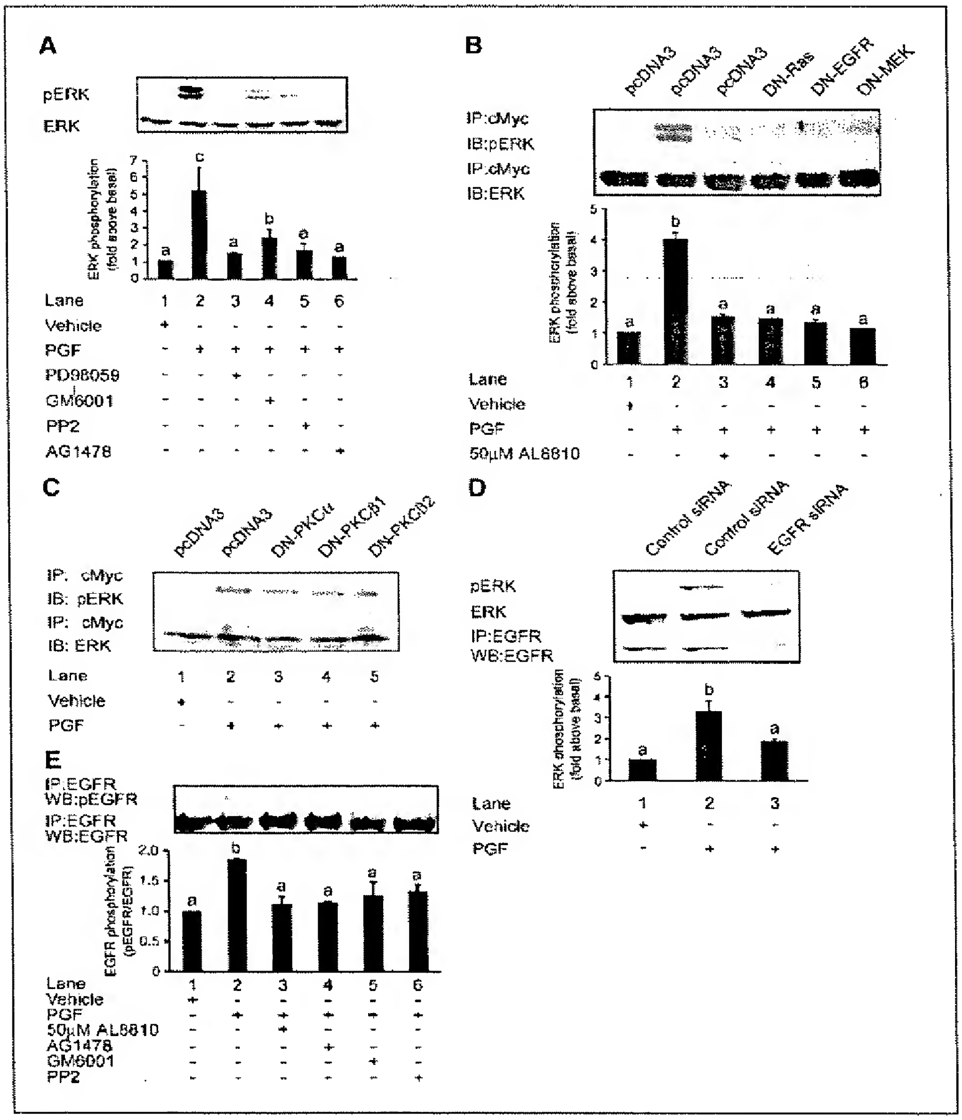

Figure 4. Representative Western blots showing activation of ERK1/2 and EGFR signaling in FPS Ishikawa cells. *A*, FPS cells were pretreated for 1 hour with inhibitors or vehicle followed by stimulation with vehicle (control; *lane 1*), 100 nmol/L $PGF_{2\alpha}$ (*lane 2*), 100 nmol/L $PGF_{2\alpha}$ and PD98059 (*lane 3*), 100 nmol/L $PGF_{2\alpha}$ and GM6001 (*lane 4*), 100 nmol/L $PGF_{2\alpha}$ and PP2 (*lane 5*), and 100 nmol/L $PGF_{2\alpha}$ and AG1478 (*lane 6*) for 10 minutes. *B*, Ishikawa FPS cells were transfected with c-Myc-tagged ERK cDNA together with pcDNA3 (control empty vector) cDNA or cDNA encoding DN-Ras, DN-EGFR, or DN-MEK. Cells were pretreated for 1 hour with 50 μmol/L AL8810 or vehicle followed by stimulation with vehicle (control), 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and 50 μmol/L AL8810 for 10 minutes. *C*, Ishikawa FPS cells were transfected with c-Myc-tagged ERK cDNA together with pcDNA3 cDNA or cDNA encoding DN-PKCα, DN-PKCβ1, and DN-PKCβ2. Cells were stimulated with vehicle (control) or 100 nmol/L $PGF_{2\alpha}$ for 10 minutes. *D*, Ishikawa FPS cells were transfected with control random siRNA or siRNA targeted against the EGFR. Cells were stimulated with vehicle or 100 nmol/L $PGF_{2\alpha}$ for 10 minutes and subjected to immunoblot analysis for phosphorylated ERK1/2, total ERK, or immunoprecipitated (*IP*) and immunoblotted (*WB*) with antisera against EGFR (*bottom*). *E*, representative Western blot showing $PGF_{2\alpha}$ transactivation of EGFR signaling in FPS cells. FPS cells were pretreated for 1 hour with inhibitors, antagonist, or vehicle followed by stimulation with vehicle (control; *lane 1*), 100 nmol/L $PGF_{2\alpha}$ (*lane 2*), 100 nmol/L $PGF_{2\alpha}$ and AL8810 (*lane 3*), 100 nmol/L $PGF_{2\alpha}$ and AG1478 (*lane 4*), 100 nmol/L $PGF_{2\alpha}$ and GM6001 (*lane 5*), and 100 nmol/L $PGF_{2\alpha}$ and PP2 (*lane 6*) for 10 minutes. After lysis, EGFR was immunoprecipitated with anti-EGFR antibody and tyrosine-phosphorylated EGFR was detected by immunoblotting with anti-phospho-EGFR antibody (*top*). The total amount of EGFR in immunoprecipitates was determined by reprobing the same blot with anti-EGFR antibody (*bottom*). Immunoblots were quantified as described in Materials and Methods. *Columns*, mean of four independent experiments; *bars*, SE. $P < 0.05$, *b* is significantly different from *a*; $P < 0.01$, *c* is significantly different from *a* and *b*. –, absence of agent; +, presence of agent.

transfected with EGFR siRNA (Fig. 4*D*, *lane 3*) was significantly reduced by 68.2 ± 1.3% compared with control transfected cells (Fig. 4*D*, *lane 2*; $P < 0.05$).

Prostaglandin $F_{2\alpha}$-FP receptor stimulation transactivates the epidermal growth factor receptor. Because ERK1/2 signaling in FPS cells could be inhibited by the EGFR protein tyrosine kinase inhibitor AG1478 as well as by transfecting FPS cells with DN-EGFR or by targeted down-regulation of EGFR with RNA interference, we investigated $PGF_{2\alpha}$-induced transphosphorylation of the EGFR in FPS cells. Ishikawa FPS cells were treated with 100 nmol/L $PGF_{2\alpha}$ or vehicle and EGFR tyrosine phosphorylation was assessed by immunoprecipitation and Western blotting. A significant increase in EGFR tyrosine phosphorylation compared with vehicle stimulation was observed in Ishikawa FPS cells stimulated with $PGF_{2\alpha}$ (Fig. 4*E*, *lane 2*). Treatment of cells with AL8810 (Fig. 4*E*, *lane 3*), AG1478 (Fig. 4*E*, *lane 4*), GM6001 (Fig. 4*E*, *lane 5*), or PP2 (Fig. 4*E*, *lane 6*) significantly reduced the $PGF_{2\alpha}$-induced EGFR tyrosine phosphorylation ($P < 0.05$).

Prostaglandin $F_{2\alpha}$-FP receptor activation induces vascular endothelial growth factor expression and secretion in Ishikawa cells. The role of $PGF_{2\alpha}$-FP receptor signaling on the expression of VEGF was investigated by quantitative RT-PCR analysis after stimulation of WT, FPS, and FPAS cells with 100 nmol/L $PGF_{2\alpha}$ or vehicle for 0, 2, 4, 8, and 24 hours. As shown in Fig. 5*A*, $PGF_{2\alpha}$ stimulation resulted in a significant fold increase in the expression of VEGF in Ishikawa FPS cells at 4, 8, and 24 hours ($P < 0.01$). However, no such increase in the expression of VEGF was observed in WT or FPAS cells. Treatment of cells with $PGF_{2\alpha}$ and AL8810 significantly reduced the $PGF_{2\alpha}$-induced expression of VEGF mRNA at 8 hours (Fig. 5*B*; $P < 0.05$), indicating that the effect of $PGF_{2\alpha}$ on VEGF is exerted via the FP receptor. We next investigated whether VEGF protein was secreted in the culture medium of FPS cells treated with 100 nmol/L $PGF_{2\alpha}$. As shown in Fig. 5*C*, $PGF_{2\alpha}$ treatment of FPS cells induced a significant elevation in VEGF protein in the culture medium at 24 hours ($P < 0.01$). Cotreatment of cells with $PGF_{2\alpha}$ and AL8810 abolished

the secretion of VEGF into the culture medium ($P < 0.05$). No significant elevation in secreted VEGF protein was observed in FPS cells at earlier time points and no elevation in VEGF protein secretion was observed in WT and FPAS cells following treatment with $PGF_{2\alpha}$ (data not shown).

The role of the EGFR and ERK1/2 signaling pathways in mediating the effects of $PGF_{2\alpha}$-FP interaction on the expression of VEGF in FPS cells was investigated by quantitative RT-PCR analysis. As shown in Fig. 5*D*, treatment of FPS cells with 100 nmol/L $PGF_{2\alpha}$ produced a 4.2 ± 0.4-fold increase in VEGF mRNA expression, which was significantly reduced by the EGFR tyrosine kinase inhibitor AG1478 ($P < 0.05$) and abolished by the selective MEK inhibitor PD98059 ($P < 0.05$). To further confirm that VEGF expression was mediated via the EGFR and Ras-MEK-ERK1/2 pathways, we used a luciferase reporter plasmid in which the full-length VEGF 5′ flanking sequence was fused to the firely luciferase coding sequence in the pGL3-basic vector (25). Additionally, a luciferase reporter without any VEGF promoter sequences (pGL3-basic) was used as a control. Cells were cotransfected with the pRL-TK (*Renilla* luciferase vector as an internal control) with either DN-Ras, DN-EGFR, and DN-MEK or an empty vector (pcDNA3.0) as a control. As shown in Fig. 5*E*, treatment of control vector-transfected cells with 100 nmol/L $PGF_{2\alpha}$ significantly elevated VEGF promoter activity by 2.5 ± 0.1-fold ($P < 0.01$). This elevation in VEGF promoter activity was abolished by cotreatment of the cells with $PGF_{2\alpha}$ and AL8810 and significantly reduced by cotransfection of cells with DN-EGFR, DN-Ras, or DN-MEK ($P < 0.01$). Cells transfected with the control luciferase reporter, without any VEGF sequence (pGL3-basic), showed no significant alteration in luciferase activity in response to treatment (Fig. 5*E*).

Prostaglandin $F_{2\alpha}$-FP receptor activation induces the expression of vascular endothelial growth factor in endometrial adenocarcinomas via transactivation of the epidermal growth factor receptor. To correlate our findings obtained using the Ishikawa FP receptor model system with $PGF_{2\alpha}$ signaling to VEGF in endometrial adenocarcinomas *in vivo*, we also used endometrial adenocarcinoma tissue explants. Endometrial adenocarcinoma tissues were treated with 100 nmol/L $PGF_{2\alpha}$ or vehicle alone for 10 minutes and EGFR phosphorylation was assessed by Western blotting. A significant increase in tyrosine phosphorylation of EGFR was observed in endometrial adenocarcinoma explants (Fig. 6*A*, *lane 2*; $P < 0.05$) in response to stimulation with $PGF_{2\alpha}$ compared with control tissue (Fig. 6*A*, *lane 1*; $P < 0.05$). Treatment of endometrial adenocarcinoma explants with AL8810 or AG1478 abolished this action of $PGF_{2\alpha}$ (Fig. 6*A*, *lanes 3* and *4*, respectively; $P < 0.05$). We next incubated endometrial adenocarcinoma explants with either vehicle or $PGF_{2\alpha}$ in the absence or presence of AL8810, AG1478, or PD98059 for 24 hours and assessed VEGF mRNA expression by quantitative RT-PCR analysis (Fig. 6*B*). $PGF_{2\alpha}$ significantly elevated the expression of VEGF in endometrial

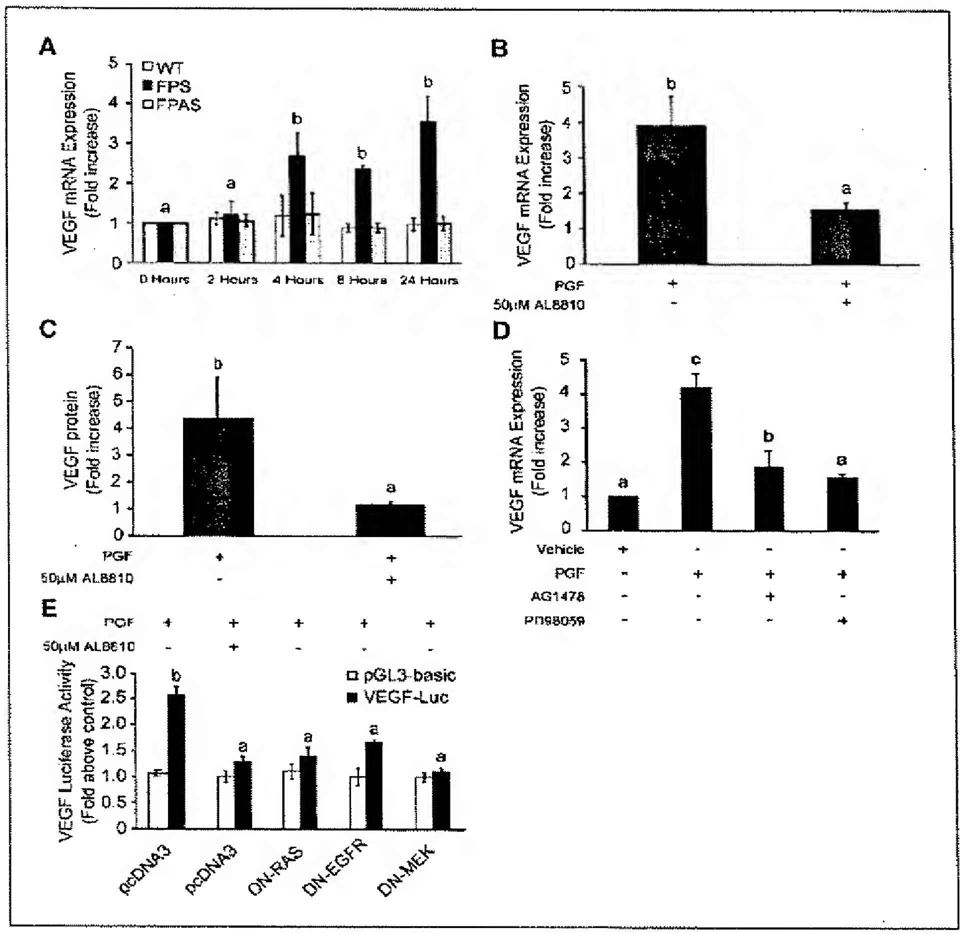

Figure 5. VEGF expression and secretion in Ishikawa cells. *A*, VEGF expression in Ishikawa WT, FPS, and FPAS cells was measured by real-time quantitative RT-PCR analysis following treatment of cells for 0, 2, 4, 8, and 24 hours with vehicle or 100 nmol/L $PGF_{2\alpha}$. *B*, VEGF expression in FPS cells at 8 hours. Cells were treated with 100 nmol/L $PGF_{2\alpha}$ in the absence or presence of FP receptor antagonist (AL8810). *C*, secretion of VEGF protein into the culture medium of FPS cells was measured by ELISA. FPS cells were treated for 24 hours with vehicle, 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and AL8810. *D*, VEGF expression in Ishikawa FPS cells in response to treatment with chemical inhibitors of EGFR and ERK signaling as measured by real-time quantitative RT-PCR analysis. Cells were treated for 8 hours with vehicle or 100 nmol/L $PGF_{2\alpha}$. In parallel, cells were treated with 100 nmol/L $PGF_{2\alpha}$ and AG1478 or 100 nmol/L $PGF_{2\alpha}$ and PD98059. *E*, VEGF promoter activity in FPS cells transfected with the full-length VEGF firefly luciferase promoter (pGL3-VEGF-luc; *filled columns*) or empty firefly luciferase control vector (pGL3-basic; *open columns*). FPS cells were transfected with pGL3-basic or pGL3-VEGF-luc and pRL-TK (*Renilla* luciferase) and cotransfected with either pcDNA3 (control empty vector cDNA) or cDNA encoding DN-Ras, DN-EGFR, and DN-MEK. Following transfection, the cells were incubated for 8 hours with either vehicle, 100 nmol/L $PGF_{2\alpha}$, or 100 nmol/L $PGF_{2\alpha}$ and AL8810, and firefly and *Renilla* luciferase activity was measured for the calculation of specific VEGF promoter activity as described in Materials and Methods. *Columns*, mean of four independent experiments; *bars*, SE. $P < 0.05$, *b* is significantly different from *a*; $P < 0.01$, *c* is significantly different from *a* and *b*. -, absence of agent; +, presence of agent.

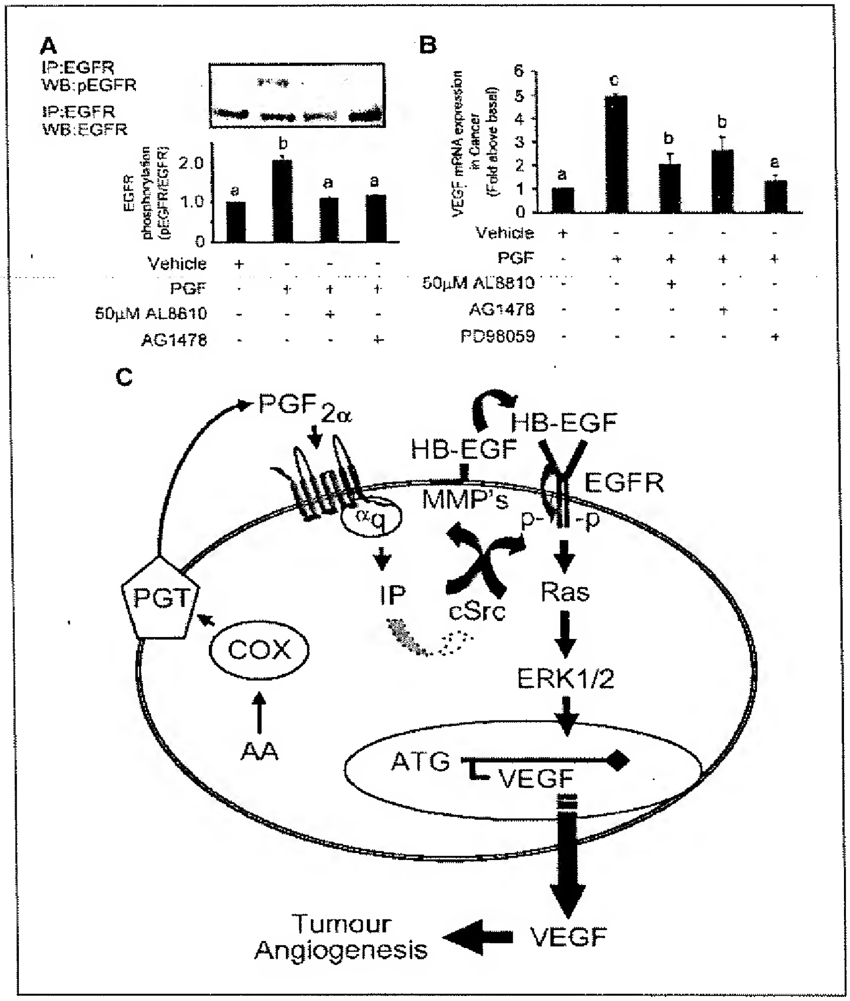

Figure 6. $PGF_{2\alpha}$ transactivation of the EGFR and activation of VEGF in endometrial adenocarcinomas. *A,* endometrial adenocarcinoma biopsy explants were pretreated for 1 hour with inhibitors or vehicle followed by stimulation with vehicle (control, *lane 1*), 100 nmol/L $PGF_{2\alpha}$ (*lane 2*), 100 nmol/L $PGF_{2\alpha}$ and AL8810 (*lane 3*), or 100 nmol/L $PGF_{2\alpha}$ and AG1478 (*lane 4*) for 10 minutes. After lysis, EGFR was immunoprecipitated with anti-EGFR antisera and tyrosine-phosphorylated EGFR was detected by immunoblotting with anti-phospho-EGFR antibody. The total amount of EGFR in immunoprecipitates was determined by reprobing the same blot with total EGFR antibody. Semiquantitative analysis of EGFR phosphorylation was determined as described in Materials and Methods. *B,* VEGF mRNA expression in endometrial adenocarcinoma in response to 100 nmol/L $PGF_{2\alpha}$ as determined by quantitative RT-PCR analysis. Endometrial adenocarcinoma explants were treated with vehicle, 100 nmol/L $PGF_{2\alpha}$, 100 nmol/L $PGF_{2\alpha}$ and AL8810, 100 nmol/L $PGF_{2\alpha}$ and AG1478, and 100 nmol/L $PGF_{2\alpha}$ and PD98059 for 24 hours. *Columns,* mean of four independent experiments; *bars,* SE. $P < 0.05$, *b* is significantly different from *a*; $P < 0.01$, *c* is significantly different from *a* and *b*. −, absence of agent; +, presence of agent. *C,* schematic representation of $PGF_{2\alpha}$-FP receptor signaling to ERK in endometrial adenocarcinoma cells. Intracellular $PGF_{2\alpha}$ produced via the actions of COX enzymes is actively transported out of the cell (*PGT*) and interacts with FP receptors in an autocrine/paracrine manner to activate inositol-1,4,5-trisphosphate. Activation of inositol phosphate can potentially initiate ERK signaling via the activation of c-Src and MMPs and transphosphorylation of the EGFR, culminating in the transcription and release of VEGF to promote tumor angiogenesis by acting on adjacent endothelial cells.

adenocarcinoma explants by 4.9 ± 0.4–fold compared with vehicle-treated tissue ($P < 0.01$). This elevation in expression of VEGF was significantly inhibited by cotreatment of tissue explants with AL8810, AG1478, and PD98059 ($P < 0.05$). These data obtained from the endometrial adenocarcinoma explants agree with the data obtained in the Ishikawa cell FP receptor model system and imply that $PGF_{2\alpha}$-FP receptor interaction can potentiate tumor angiogenesis in endometrial adenocarcinomas by enhancing the expression of proangiogenic factors, such as VEGF, following the activation of the EGFR and ERK1/2 pathways mediated by the actions of c-Src and MMPs (Fig. 6*C*).

Discussion

A relationship between $PGF_{2\alpha}$ and vascular dysfunction of the endometrium has been described (13, 27, 28). Elevated $PGF_{2\alpha}$ concentrations of up to 2 µmol/L have been reported in the menstrual fluid of women with dysmenorrhea (7). This concentration is 10-fold greater than that observed for normal women with painless periods (7). In addition, a correlation between $PGF_{2\alpha}$ concentration and excessive menstrual blood loss has also been reported in patients diagnosed with menorrhagia (13, 28, 29). Recent findings in our laboratory have shown elevated COX-2 expression and synthesis and signaling of prostanoids, including $PGF_{2\alpha}$, in human endometrial adenocarcinomas (4). Moreover, we have recently reported elevated expression of the FP receptor in endometrial adenocarcinomas compared with normal endometrium and ascertained a role for $PGF_{2\alpha}$-FP receptor signaling in tumorigenesis by enhancing proliferation of endometrial adenocarcinoma cells (6). In the present study, we show colocalized and elevated expression of FP receptor and VEGF within the glandular epithelial cells and endothelial cells lining the blood vessels in endometrial adenocarcinomas. These findings suggest a possible paracrine regulation of tumor vascular function by $PGF_{2\alpha}$ via the FP receptor.

To elucidate the molecular mechanisms whereby $PGF_{2\alpha}$, via the FP receptor, could modulate the expression of proangiogenic factors, such as VEGF, and thereby promote tumor angiogenesis, we overexpressed FP receptor in Ishikawa cells by introducing the FP receptor cDNA in the sense and antisense orientations. This was done to raise the levels of FP receptor in the Ishikawa cell line to the levels observed in endometrial adenocarcinomas to create a model system that best represents FP receptor signaling in endometrial adenocarcinoma *in vivo*. Furthermore, because at

least nine prostanoid receptors have been detected to date, any attempt to dissect out the signaling of a given receptor is made difficult due to the multiplicity of different signaling pathways and complex networks of cross-talk between them. Hence, our model presented here provides a unique opportunity to dissect out the signaling of a single receptor (i.e., the FP receptor). Elevated FP receptor expression in Ishikawa FPS cells compared with WT and FPAS cells was confirmed by Western blot analysis, immunofluorescence microscopy, and accumulation of inositol phosphate and localized to the plasma membrane, indicating that the overexpressed receptors were trafficked to the correct intracellular compartment. The expression of FP receptor in FPS cells also correlated closely with levels of expression of FP receptor in endometrial adenocarcinomas, and the increase in inositol phosphate accumulation in FPS cells compared with WT and FPAS cells was similar to that observed in endometrial adenocarcinoma explants stimulated with $PGF_{2\alpha}$, which we reported previously (6). We therefore propose that the Ishikawa FP receptor cell line is a good model system for investigating the molecular signal transduction pathways mediating the role of $PGF_{2\alpha}$ in endometrial adenocarcinoma cells via the FP receptor, which may be involved in the development and/or progression of endometrial tumors *in vivo*.

The integrated response to GPCR coupling results in activation of numerous effector signaling pathways, including the MAPK pathway (30). The MAPK pathway is a key signaling mechanism that regulates many cellular functions, such as growth, differentiation, and transformation (30, 31). The upstream component of the ERK-MAPK pathway is the GTPase Ras, which activates the serine/threonine kinase Raf that in turn phosphorylates and activates ERK1/2 (32, 33). We examined $PGF_{2\alpha}$ activation of the downstream MAPK cascades (ERK, p38, and JNK). We found that, within our experimental paradigms, $PGF_{2\alpha}$ induced a rapid increase in ERK (but not p38 or JNK) phosphorylation. This $PGF_{2\alpha}$-induced effect was significantly elevated in FPS cells compared with WT and FPAS cells and was mediated via the FP receptor, because ERK1/2 phosphorylation in FPS cells could be abolished by cotreatment of the cells with the specific FP receptor antagonist (AL8810). Furthermore, we have shown that $PGF_{2\alpha}$-induced phosphorylation of ERK1/2 in FPS cells occurs in a c-Src-, MMP-, EGFR-, and Ras-dependent manner, because ERK1/2 phosphorylation could be inhibited with specific chemical inhibitors of c-Src (PP2), MMP (GM6001), and EGFR kinase (AG1478) or by cotransfection of FPS cells with DN-EGFR, DN-Ras, and DN-MEK or by siRNA targeted against the EGFR. Interestingly, we observed that $PGF_{2\alpha}$-induced phosphorylation of ERK1/2 occurred independently of PKC activation.

Recent data in our laboratory have indicated that transphosphorylation of the EGFR by $PGF_{2\alpha}$ is crucial for transducing mitogenic signaling and cell growth (6). Several mechanisms are proposed for the transactivation of EGFR by GPCRs (34–36). One of these mechanisms involves the activation of transmembrane MMP and extracellular release of heparin-binding EGF (HB-EGF) from its latent membrane-spanning precursor in the plasma membrane. Once cleaved, the HB-EGF ligand can associate with and activate the EGFR and induce ERK1/2 activation. Alternatively, activation the EGFR can also occur via the activation of the c-Src family of nonreceptor tyrosine kinases (34–36). We found that the effect of $PGF_{2\alpha}$ on the transphosphorylation of EGFR and ERK activation in FP receptor–expressing Ishikawa cells seems to be mediated by c-Src as well as MMPs. Recently, Guerrero et al. (37) have also shown transactivation of the EGFR by c-Src as well as MMPs, suggesting that EGFR signaling can be mediated via an intracellular c-Src-dependent as well as extracellular MMP-dependent mechanism coactivated in the same cell in a cell context–dependent manner.

Tumor growth is dependent on angiogenesis. To sustain and facilitate growth in an environment where oxygen and nutrients are limiting, cancer cells produce a variety of factors, including VEGF, to create a proangiogenic environment and promote neovascularization (38, 39). VEGF is a heparin-binding glycoprotein having potent angiogenic, mitogenic, and vascular permeability-enhancing properties that are specific for endothelial cells (40). Our data show that $PGF_{2\alpha}$-FP receptor activation results in elevated expression and secretion of VEGF in Ishikawa FP cells and endometrial adenocarcinoma explants. Our approach using chemical inhibitors of signal transduction or DN mutant isoforms of signaling effectors confirm further the involvement of the EGFR to ERK1/2 signaling in the activation of VEGF expression and secretion following $PGF_{2\alpha}$-FP receptor interaction. Elevated VEGF expression and secretion would in turn exert a paracrine function on endothelial cells to promote angiogenesis, thus enhancing blood flow to the tumor and creating an environment to sustain tumor growth. Such a mechanism has been proposed for the action of another prostanoid (i.e., prostaglandin E_2), via its interaction with the EP2 receptor (20, 38, 41, 42).

Taken together, our findings using the Ishikawa FP receptor model system and which have been confirmed using endometrial adenocarcinoma explants show for the first time that $PGF_{2\alpha}$-FP receptor interaction can promote the transcription and release of a potent proangiogenic factor (i.e., VEGF) via the transactivation of the EGFR leading to ERK1/2 signaling as depicted schematically in Fig. 6C. Moreover, we propose that targeted inhibition of FP receptor, EGFR, and ERK function in endometrial carcinomas could effectively block the signaling and transcription of target genes, such as VEGF, associated with angiogenesis. High EGFR levels in endometrial cancer have been correlated with poor histopathologic grading, greater invasiveness, and reduced patient survival (43, 44). Blockade of EGFR signaling with an orally active EGFR tyrosine kinase inhibitor has been used successfully in carcinomas of nude mice to inhibit tumor angiogenesis by reducing VEGF expression (45). Similarly, the use of an orally active inhibitor of the Ras-ERK cascade has been proven efficacious in treatment of metastatic melanoma (46). Recently, Torrance et al. (47) have shown that a combinatorial approach using a nonselective COX enzyme inhibitor in combination with an inhibitor of EGFR kinase can reduce polyp formation in $APC^{\Delta716}$ mice more effectively than either compound on their own (47). In light of this latter finding and our observations presented herein, an ERK inhibitor or EGFR tyrosine kinase inhibitor in combination with a FP receptor antagonist may be of clinical relevance as an efficacious therapy for women with endometrial adenocarcinoma. Our data may also have relevance for other endometrial pathologies that are associated with disturbed vascular function and elevated prostanoid biosynthesis, such as menorrhagia, dysmenorrhea, and endometriosis.

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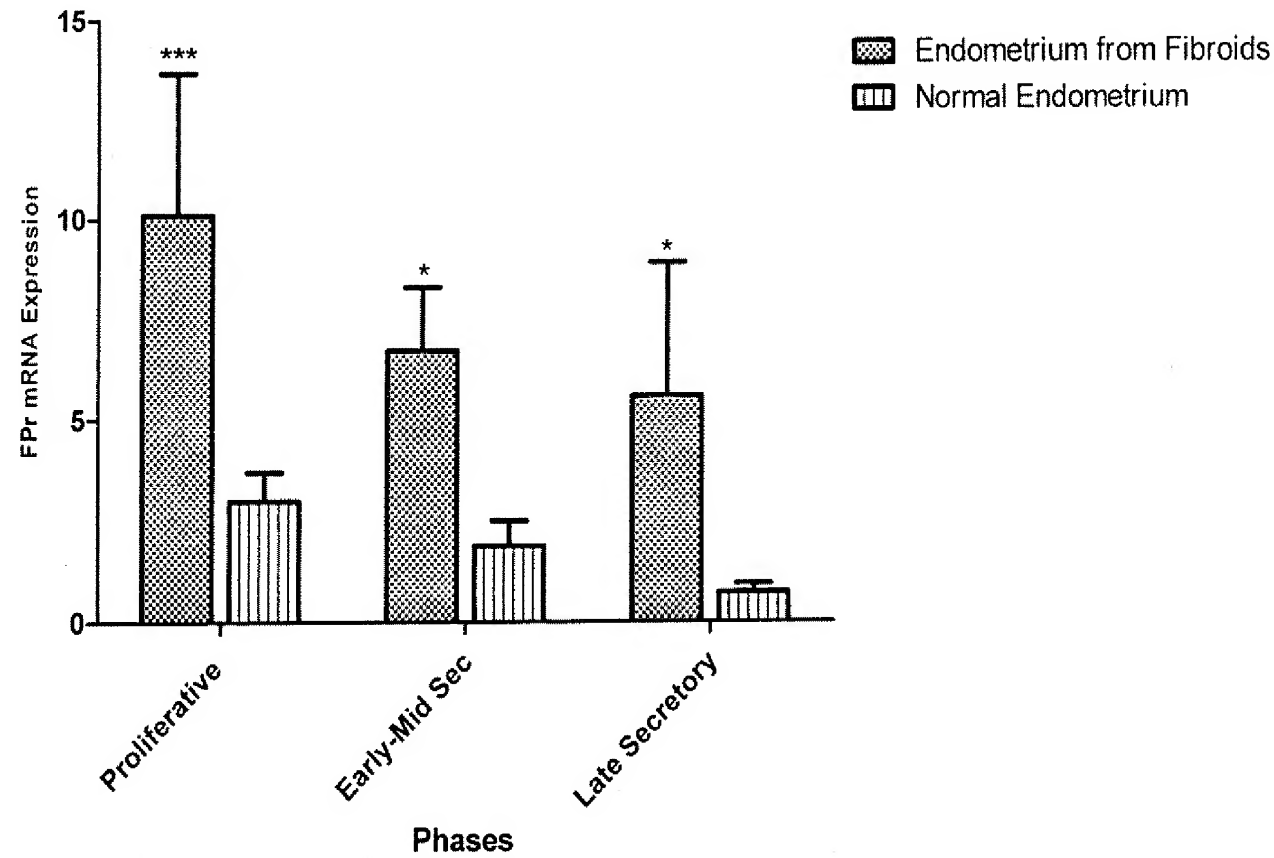
References

1. Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063–73.
2. Lipsky PE. Role of cyclooxygenase-1 and -2 in health and disease. Am J Orthop 1999;28:8–12.
3. Sales KJ, Jabbour HN. Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. Reproduction 2003;126:559–67.
4. Jabbour HN, Milne SA, Williams ARW, Anderson RA, Boddy SC. Expression of COX-2 and PGE synthase and synthesis of PGE_2 in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. Br J Cancer 2001;85:1023–31.
5. Sales KJ, Katz AA, Davis M, et al. Cyclooxygenase-2 expression and prostaglandin E2 synthesis are up-regulated in carcinomas of the cervix: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. J Clin Endocrinol Metab 2001;86:2243–9.
6. Sales KJ, Milne SA, Williams AR, Anderson RA, Jabbour HN. Expression, localization, and signaling of prostaglandin $F_{2\alpha}$ receptor in human endometrial adenocarcinoma: regulation of proliferation by activation of the epidermal growth factor receptor and mitogen-activated protein kinase signaling pathways. J Clin Endocrinol Metab 2004;89:986–93.
7. Lumsden MA, Kelly RW, Baird DT. Primary dysmenorrhoea: the importance of both prostaglandins E_2 and $F_{2\alpha}$. Br J Obstet Gynaecol 1983;90:1135–40.
8. Hofer G, Bieglmayer C, Kopp B, Janisch H. Measurement of eicosanoids in menstrual fluid by the combined use of high pressure chromatography and radioimmunoassay. Prostaglandins 1993;45:413–26.
9. Chandrasekharan NV, Dai H, Roos KL, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A 2002;99:13926–31.
10. Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001;108:25–30.
11. Chan BS, Satriano JA, Pucci M, Schuster VL. Mechanism of prostaglandin E_2 transport across the plasma membrane of HeLa cells and *Xenopus* oocytes expressing the prostaglandin transporter "PGT". J Biol Chem 1998;273:6689–97.
12. Abramovitz M, Boie Y, Nguyen T, et al. Cloning and expression of a cDNA for the human prostanoid FP receptor. J Biol Chem 1994;269:2632–6.
13. Smith SK, Abel MH, Kelly RW, Baird DT. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. Br J Obstet Gynaecol 1981;88:434–42.
14. Rees MC, Anderson AB, Demers LM, Turnbull AC. Prostaglandins in menstrual fluid in menorrhagia and dysmenorrhoea. Br J Obstet Gynaecol 1984;91:673–80.
15. Folkman J. Angiogenesis and angiogenesis inhibition: an overview. EXS 1997;79:1–8.
16. Jain RK, Booth MF. What brings pericytes to tumor vessels? J Clin Invest 2003;112:1134–6.
17. Guidi AJ, Abu-Jawdeh G, Tognazzi K, Dvorak HF, Brown LF. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in endometrial carcinoma. Cancer 1996;78:454–60.
18. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
19. Berg KA, Clarke WP, Sailstad C, Saltzman A, Maayani S. Signal transduction differences between 5-hydroxytryptamine type 2a and type 2c receptor systems. Mol Pharmacol 1994;46:477–84.
20. Sales KJ, Maudsley S, Jabbour HN. Elevated prostaglandin EP2 receptor in endometrial adenocarcinoma cells promotes vascular endothelial growth factor expression via cyclic 3',5'-adenosine monophosphate-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase 1/2 signaling pathways. Mol Endocrinol 2004;18:1533–45.
21. Levi NL, Hanoch T, Benard O, et al. Stimulation of Jun N-terminal kinase (JNK) by gonadotropin-releasing hormone in pituitary α t3-1 cell line is mediated by protein kinase C, c-Src, and cdc42. Mol Endocrinol 1998;12:815–24.
22. Benard O, Naor Z, Seger R. Role of dynamin, src, and ras in the protein kinase C-mediated activation of ERK by gonadotropin-releasing hormone. J Biol Chem 2001;276:4554–63.
23. Bonfil D, Chuderland D, Kraus S, et al. Extracellular signal-regulated kinase, Jun N-terminal kinase, p38, and c-Src are involved in gonadotropin-releasing hormone-stimulated activity of the glycoprotein hormone follicle-stimulating hormone β-subunit promoter. Endocrinology 2004;145:2228–44.
24. Harris D, Bonfil D, Chuderland D, et al. Activation of MAPK cascades by GnRH: ERK and Jun N-terminal kinase are involved in basal and GnRH-stimulated activity of the glycoprotein hormone LHβ-subunit promoter. Endocrinology 2002;143:1018–25.
25. Shi Q, Le X, Abbruzzese JL, et al. Constitutive sp1 activity is essential for differential constitutive expression of vascular endothelial growth factor in human pancreatic adenocarcinoma. Cancer Res 2001;61:4143–54.
26. Griffin BW, Klimko P, Crider JY, Sharif NA. AL-8810: a novel prostaglandin $F_{2\alpha}$ analog with selective antagonist effects at the prostaglandin $F_{2\alpha}$ (FP) receptor. J Pharmacol Exp Ther 1999;290:1278–84.
27. Lundstrom V, Green K. Endogenous levels of prostaglandin $F_{2\alpha}$ and its main metabolites in plasma and endometrium of normal and dysmenorrheic women. Am J Obstet Gynecol 1978;130:640–6.
28. Hofmann GE, Rao CV, Barrows GH, Sanfilippo JS. Topography of human uterine prostaglandin E and $F_{2\alpha}$ receptors and their profiles during pathological states. J Clin Endocrinol Metab 1983;57:360–6.
29. Adelantado JM, Rees MC, Lopez Bernal A, Turnbull AC. Increased uterine prostaglandin E receptors in menorrhagic women. Br J Obstet Gynaecol 1988;95:162–5.
30. Naor Z, Benard O, Seger R. Activation of MAPK cascades by G-protein-coupled receptors: the case of gonadotropin-releasing hormone receptor. Trends Endocrinol Metab 2000;11:91–9.
31. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. Adv Cancer Res 1998;74:49–139.
32. Wells A. EGF receptor. Int J Biochem Cell Biol 1999;31:637–43.
33. Kranenburg O, Moolenaar WH. Ras-MAP kinase signaling by lysophosphatidic acid and other G-protein-coupled receptor agonists. Oncogene 2001;20:1540–6.
34. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. Nature 1996;379:557–60.
35. Luttrell LM, Daaka Y, Lefkowitz RJ. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol 1999;11:177–83.
36. Maudsley S, Pierce KL, Zamah AM, et al. The β2-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. J Biol Chem 2000;275:9572–80.
37. Guerrero J, Santibanez JF, Gonzalez A, Martinez J. EGF receptor transactivation by urokinase receptor stimulus through a mechanism involving src and matrix metalloproteinases. Exp Cell Res 2004;292:201–8.
38. Tsujii M, Kawano S, Tsuji S, et al. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705–16.
39. Sales KJ, Katz AA, Howard B, et al. Cyclooxygenase-1 is up-regulated in cervical carcinomas: autocrine/paracrine regulation of cyclooxygenase-2, PGE receptors and angiogenic factors by cyclooxygenase-1. Cancer Res 2002;62:424–32.
40. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306–9.
41. Seno H, Oshima M, Ishikawa T, et al. Cyclooxygenase-2 and prostaglandin E2 receptor EP2-dependent angiogenesis in APCΔ716 mouse intestinal polyps. Cancer Res 2002;62:506–11.
42. Sonoshita M, Takaku K, Sasaki N, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in APCΔ714 knockout mice. Nat Med 2001;7:1048–51.
43. Scambia G, Benedetti Panici P, Ferrandina G, et al. Significance of epidermal growth factor receptor expression in primary human endometrial cancer. Int J Cancer 1994;56:26–30.
44. Niikura H, Sasano H, Matsunaga G, et al. Prognostic value of epidermal growth factor receptor expression in endometrioid endometrial carcinoma. Hum Pathol 1995;26:892–6.
45. Kedar D, Baker CH, Killion JJ, Dinney CP, Fidler IJ. Blockade of the epidermal growth factor receptor signaling inhibits angiogenesis leading to regression of human renal cell carcinoma growing orthotopically in nude mice. Clin Cancer Res 2002;8:3592–600.
46. Collisson EA, De A, Suzuki H, Gambhir SS, Kolodney MS. Treatment of metastatic melanoma with an orally available inhibitor of the Ras-Raf-MAPK cascade. Cancer Res 2003;63:5669–73.
47. Torrance CJ, Jackson PE, Montgomery E, et al. Combinatorial chemoprevention of intestinal neoplasia. Nat Med 2000;6:1024–8.

Annex 4: Fibroid data



15
10
5
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FPr mRNA Expression
Proliferative
Early-Mid Sec
Late Secretory
Phases
Endometrium from Fibroids
Normal Endometrium